ATTACHMENT
to the Decision of the Board
The Eurasian Economic Commission
from «» 20, No.

CHANGES,
introduced in Section 20 of Chapter II of the Unified Sanitary-epidemiological and hygienic requirements for products (goods), subject to sanitary and epidemiological supervision (control)

Section 20 Unified sanitary epidemiological and hygienic requirements for products (goods) subject to sanitary-epidemiological supervision (control), outline in the next edition:

"Section 20. Requirements for disinfectants

1 area of use

These requirements apply to disinfection means in the form of various preparative forms, designed for professional application, retail sale to the population (except means used in veterinary and agricultural) (codes TNEC FEA of the EEA 3808).

2. Terms and definitions

For the purposes of this section, concepts that mean the following:

"Safety of disinfectants" - acceptable probability of the absence of a harmful effect of their impact on people and environment under certain conditions and regimes;

"Disinsection means" means used for reduction to an acceptable level or destruction of arthropods on / in objects of the external environment;

"Disinfection expertise" is the procedure for reviewing and evaluation of materials characterizing the disinfectant, including the results of laboratory, instrumental, field research and testing of chemical composition, safety, target effectiveness, as well as the accompanying disinfectant normative, methodical and instructive documentation, carried out in order to protect the life or health of citizens, prevention of actions that mislead consumers.
The result of the disinfection expert examination is an expert conclusion;

"Disinfection means" means intended for holding disinfection (disinfecting facilities), pre-sterilization cleaning, sterilization (sterilization means, products), pest control (insecticidal, pediculicidal, acaricides), deratization (deratant), as well as repellent means;

"Disinfectants (disinfectants)" - means used to reduce to an acceptable level or destruction of microorganisms in / on environmental objects;

"Disinfectant for high level disinfection endoscopes "- a means to ensure the death of vegetative forms

bacteria (including mycobacteria), fungi, shell and non-enveloped viruses and a certain amount of spores;

"Active substance (substance)" - chemical and / or biological substances that are part of the disinfectants,

providing the target efficiency »;

"Deratization means" means the means used for

reduce the number of rodents to an acceptable level or their

destruction;

"Treatment of disinfectant" - life cycle

Disinfectant means, including development, registration,

production, transportation, implementation, application,

disposal and / or destruction;

"Assessment of real danger" - the definition and evaluation of negative

effects of the disinfectant and the conditions (technology) of its

use of human health and environmental

long-term and short-term contact;

"Preparative form" - ready for use by the target

a disinfectant consisting of an active

substances (substances) or mixtures of active substances and

functional components;

"Mode of application" - a set of factors, conditions,

technology of disinfection means, providing

achievement of target efficiency and safety;

"Repellent means" means intended for

scaring of arthropods or rodents;

"Means of pre-sterilization cleaning » - facilities,

designed to remove contaminants from medical products,

preventing sterilization or reducing its effectiveness;

"Waiting period" - the time period from the moment of completion

disinfection treatment of the facility, premises

(territory) prior to its safe use;

"Sterilizing agents" means the means intended for

destruction of all kinds of microorganisms, including bacteria,

viruses, prions, fungi at all stages of development;

"Effectiveness of the disinfectant (effectiveness disinfection) "- an absolute or relative indicator, characterizing the achieved level of sterilization, disinfection, disinsection, deratization.

3. Requirements (criteria) for safety and efficiency disinfectants

Safety and effectiveness of disinfectants is ensured by a set of requirements for physicochemical, toxicological and biological indicators.

For definitions security preparatory forms disinfection means are evaluated:

a) the chemical and physical properties of disinfectants, including their volatility, stability, compatibility with other connections (information is provided by the manufacturer);

b) FAO / WHO data (if available), or the European Union, or the United States Environmental Protection Agency (EPA) the dangers of imported disinfectants;

c) Toxicological characteristic components formulation - fillers, emulsifiers, stabilizers, solvents and others, with indication for each of them of the current standard, or registration in the REACH system, or registration CAS numbers, IURA names;

d) acute toxicity when brought into the stomach;

e) Acute toxicity when applied to the skin;

e) acute inhalation toxicity;

g) acute toxicity when administered into the abdominal cavity;

h) irritating effect on the skin and mucous membranes of the eyes;

i) sensitizing action;

j) cumulative effect;

k) skin-resorptive action;

m) subacute inhalation toxicity;

n) subacute toxicity with intragastric intake;

o) chronic toxicity with intragastric intake;

n) influence on organoleptic properties (smell, taste) of water;

p) hygienic standards (MPC / MPLS) DV in the working air zones, in the atmospheric air of populated areas, in water objects [1] ;

c) hygienic standards (MPC) of transformation products DV, harmful impurities and transformation products under the action of DV in water bodies of water objects (for means of disinfection of all types of water);

[1] hygienic rationing is not required for active substances that are not are classified as hazardous by parameters of acute toxicity with intragastric, cutaneous, inhalation intake into the body, by the ability to possess irritating, skin-resorptive, sensitizing, reprotoxic , mutagenic, carcinogenic action ";

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t) data on long-term effects (mutagenic, carcinogenic, embryotoxic, teratogenic, influence on reproductive and endocrine system);

y) a real hazard in the recommended modes of application;

f) the specific effectiveness of the formulations disinfection.

As active substances, it is prohibited to use chemicals subject to Rotterdam Convention on the Prior Informed Consent Procedure and Annexes A and B of the Stockholm Convention on Persistent Organic Pollutants pollutants.

Treating skin antiseptics and treatment agents injecting and operating fields to disinfectants or medicines, as well as examination and registration of skin antiseptics and tools for the treatment of injection and operating fields, carried out at accordance from national legislation of the Member States.

Normative indicators toxicity and security,

physicochemical indicators of disinfectants are accepted in accordance with Appendix 20.1.

The study and assessment of toxicity and safety conducts in accordance with the regulatory documentation given in Appendix 20.1.

The study and evaluation of the effectiveness of disinfectants shall be carried out in accordance with Appendix 20.2.

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4. Disinfection expert examination

Disinfection expert examination for the purpose of state registration is based on the following principles:

mandatory;

scientific validity (evidence);

independence of experts in the implementation of their powers;

completeness of the examination;

ensuring the confidentiality of the materials in question;

pay for the examination.

Disinfectological examination of disinfectants carry out the organization of authorized bodies, accredited according to established order.

The order of organization and conduct of disinfectological expertise is determined in accordance with the national legislation of the Member States.

For examination of disinfection means by the manufacturer (supplier, registrant) provides a dossier that includes:

formulation of the product;

technical conditions or other normative legal document on manufactured products, manufacturer's standard (for products manufactured in the territory of the Member States);

Specification (for imported products);

instruction (project) for the application of the remedy, including general information about the product, its purpose, active ingredients, toxicological characteristics, preparation of working solutions,

recommendations for use, safety and first aid measures in cases of poisoning with a disinfectant, methods recycling;

methods of quality control means, including the method of control active ingredient;

certificate of stability / expiration dates of the funds;

sample label;

toxicological characteristics of the active substance, the main components and the formulation, in general, hygienic standards in environmental objects (water, air, soil) [2] ;

methods of controlling the active substance and / or its products transformation in environmental objects (water, air, soil);

Material Safety Data Sheet (MSDS) and / or security sheet in full, including remote Effects (mutagenic activity, teratogenic act, carcinogenic and embryotoxic action, gonadotoxic act);

data on the safety of nanomaterials in the case of their introduction in the composition of the disinfectant;

the results of a study of targeted efficiency and safety;

[2] hygienic rationing is not required for active substances that are not are classified as hazardous by parameters of acute toxicity with intragastric, cutaneous, inhalation intake into the body, by the ability to possess irritating, skin-resorptive, sensitizing, repotoxic, mutagenic, carcinogenic action ";

measures for the safe handling of disinfectants,
specified in regulatory legal acts on products or
in the safety data sheet;

sample of a disinfectant preparation
in the manufacturer's packaging with an act of sampling (sampling) for
products produced in the territory of the Member States;

standard sample of active disinfectant
facilities.

As a result of the disinfectional examination of disinfection
an expert opinion on the possibility of
state registration of a disinfectant for
customs territory of the Eurasian Economic Union,
containing the following information:

the name of the disinfectant (its preparative
the form);

manufacturer of the formulation;

manufacturer acting matter (s)
disinfectant;

toxicological characteristics of the disinfectant and
its working solutions and forms ready for use;

main results of chemical and analytical control, assessment
target efficacy and safety of the disinfectant;

purpose of the disinfectant;

the area of application of the disinfectant;

recommendations for state registration at the customs
territory of the Union.

AT the case lack of necessary materials for
disinfectological examination of the disinfectant,

lack of targeted effectiveness, identification of unavoidable negative toxicological and hygienic properties or obtaining negative results during the pilot research, etc., an informed conclusion is The impossibility of state registration of disinfection facilities.

5. Packaging and labeling

Packaging for disinfectants is performed from materials that ensure the safety of products and excluding opportunity pollution disinfection environment in their storage, transportation and application.

Packaging for packing aggressive disinfectants, including number with a pH of less than 2.0 units. and more than 11.5 units, must be supplied A special device for their safe pouring.

Transport marking is applied directly to the packaging printing machines, either by stencil or label stickers (stickers). It should contain: the name of the facility, manipulation marks, classification code, information for with the obligatory indication of the batch number, the date manufacture (month, year) and warranty period of storage.

Annex 20.1 to Section 20
Unified sanitary-epidemiological and hygienic requirements for products (goods), subject to sanitary and epidemiological supervision (control)

Normative indicators of toxicity and safety disinfectants

1. Indicators of toxicity and safety of disinfectants

1.1. Disinfectants

Appointment facilities	Subjects indicators	Standards		Allowed application of
		Value Indicator	Classification judgment *	
1.1.1. Means for disinfection of surfaces of premises, rigid furniture, devices and devices, linen, shoes, tableware, laboratory and from under the vydeleny, toys (except soft), sanitation equipment, items of care for patients, secretions, garbage chutes, garbage collection (treatment methods: wiping, irrigation, soaking and immersion) , waste				
1.1.1.1. Disinfectants means in the form of concentrate, liquid, powder, granules, tablets, gel etc.	Acute toxicity with introduction into the stomach (DL_{50}, mg / kg)	> 150 (> 50 - ≤ 2000)	3-4 (3-4)	** Specialists with use of PPE (respirators, rubber gloves)
		> 5000 (> 2000)	4 (5)	Specialists and population in everyday life

Appointment facilities	Subjects indicators	Standards		Allowed application of
		Value Indicator	Classification judgment *	
	Acute toxicity with application to the skin (DL_{50}, mg / kg)	> 500	3-4	For specialists with PPE (respirators, rubber gloves)
		> 200 - ≤ 2000	(3-4)	
		> 2000 (> 2000)	4 (5)	Specialists and population in everyday life
	Acute inhalation danger in saturating The concentration of vapor (C_{20}) at 20 ° C	C_{20} - clinic	2	For specialists with PPE (respirators, protective glasses, rubber gloves)
		$C_{20} \geq Lim_{ac}$	3	For specialists with PPE (respirators, protective glasses, rubber gloves)
		$C_{20} < Lim_{ac}$	4	Specialists and population in everyday life
	Acute irritating / corroding effect on skin, scores (erythema, edema)	> 4 (≥ 2.3)	1-2 (1-2)	For specialists with PPE (respirators, protective glasses, rubber gloves)
		2.1-4.0 (≥ 1.5 - <2.3)	3 (3)	Specialists and population in life with the use of PPE (latex gloves)
		0 - 2.0 (<1.5)	4 (not classified)	Specialists and population in life with the use of PPE (latex gloves)
	Acute irritating action on the eyes, scores	more than 4	1-3	For specialists with PPE (respirators, protective glasses, rubber gloves)
		0-3	4-5	Specialists and population in everyday life
	Sensitizing act	moderate / weak (moderate / low)	3A / 3B (1B)	Specialists and population in life with the use of PPE

Appointment facilities	Subjects indicators	Standards		Allowed application of
		Value Indicator	Classification judgment *	
	(skin / respiratory)			(latex gloves)
		lack of effect (no effect)	4 (not classified)	Specialists and population i everyday life
1.1.1.2. Working solutions disinfecting means	Acute irritating / corroding effect on skin, scores (erythema, edema)	2.1-4.0 (≥ 1.5 - <2.3)	3 (3)	For specialists with PPE (respirators, protective glasses, rubber gloves)
		0 - 2.0 (<1.5)	4 (not classified)	Specialists and population i everyday life
	Irritant effect on skin during repeated applications (0.5-1 months)	moderate / weak	It is not classified to specialists using	PPE (rubber gloves)
		lack of effect		Specialists and population i everyday life
	Skin Resorptive action (21/28 days)	effect	It is not classified to specialists using	PPE (rubber gloves)
		lack of effect		Specialists and population i everyday life
	Acute irritating action on the eyes, scores	4-6	3	For specialists with PPE (safety goggles, latex gloves)
		0-3	4-5	Specialists and population i everyday life

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Appointment facilities	Subjects indicators	Standards		Allowed application of
		Value Indicator	Classification judgment *	
	Inhalation hazard in modes of application: Acute toxic zone actions:	less than 1	1	According to the epidemo-sheets with PPE application (gas mask / respirators, safety glasses, rubber gloves) For specialists with PPE (respirators, protective glasses, rubber gloves) in no patients Specialists in the absence patients Specialists and population i everyday life
		1-3	2	
		3.1-10	3	
		more than 10	4	
	Subacute Toxic Zone actions	less than 10	It is not classified in the absence of specialists.	patients
		more than 10		Specialists in the presence c patients and the population everyday life
	Safety assessment residual amounts of DS on dishes (optional):			

	- cytotoxicity (CCL)	decrease CTD50 (but not more than 1)		No limits
	bull spermatozoa	toxicity index	not classified	No limits
	- hemolysis of erythrocytes	70-120% not more than 2%		No limits
	- pyrogenicity	lack of effect		No limits
	Conformity assessment content	C / MPC (OBUV) r.z. > 1	1-4	For specialists with PPE (respirators, protective

Appointment facilities	Subjects indicators	Standards Value Indicator	Classification judgment *	Allowed application of
	solution (C) in the air hygienic standards (conducted with necessity)			glasses, rubber gloves) in the absence of patients
		C / MPC (OBUV) r.z. ≤1	1-4	Specialists in the absence patients
		C / MAC (OBUV) as. ≤1	3-4	Specialists in the presence o patients and the population everyday life
1.1.2. Disinfectants in aerosol form, intended for air and surface treatment premises by a volumetric method, and also surfaces - by directed aerosols				
1.1.2.1. Funds in aerosol form	Inhalation hazard in application mode:	less than 1	1	According to the epidemo-sheets with PPE application
	Acute toxic zone actions:	1-3	2	(gas mask / respirators, safety glasses, rubber gloves) For specialists with PPE (respirators, protective glasses, rubber gloves) in
		3.1-10	3	no patients Specialists in the absence
		more than 10	4	patients To specialists
	Subacute Toxic Zone actions	less than 10	It is not classified in the absence of specialists.	patients
		more than 10		Specialists in the presence o patients

Appointment facilities	Subjects indicators	Standards Value Indicator	Classification judgment *	Allowed application of
	Acute irritating / corroding effect on skin, scores (erythema, edema)	2.1 - 4.0 (≥ 1.5 - <2.3)	3 (3)	For specialists with PPE (respirators, rubber gloves)
		0 - 2.0 (<1.5)	4 (not classified)	Specialists and population i everyday life
	Acute irritating	4-6	3	For specialists with

	action on the eyes, scores			PPE (respirators, protective glasses, rubber gloves)
		0-3	4-5	To specialists
	Sensitizing effect (skin / respiratory)	moderate / weak (moderate / low)	3A / 3B (1B)	For specialists with PPE (rubber gloves)
		lack of effect (no effect)	4 (not classified)	Specialists and population in everyday life
	Conformity assessment content solution (C) in the air hygienic standards (conducted with necessity)	C / MPC (OBUV) r.z. > 1	1-4	For specialists with PPE (respirators, protective glasses, rubber gloves) in the absence of patients
		C / MPC (OBUV) r.z. ≤1	1-4	Specialists in the absence patients
		C / MAC (OBUV) as. ≤1	3-4	Specialists in the presence c patients
1.1.2.2. Disinfectants means for disinfection of waste in the form of a concentrate, liquid, powder, granules, tablets, gel etc.	Acute toxicity with introduction into the stomach (DL 50 , mg / kg)	> 150 (> 50 - ≤ 2000)	3-4 (3-4)	For specialists with PPE (respirators, protective glasses, rubber gloves)
		> 2000 (> 2000)	4 (5)	Specialists and population in everyday life
	Acute toxicity with application to the skin (DL 50 , mg / kg)	> 500 (> 200 - ≤ 2000)	3-4 (3-4)	For specialists with PPE (respirators, protective glasses, rubber gloves)

Appointment facilities	Subjects indicators	Standards Value Indicator	Classification judgment *	Allowed application of
		> 2000 (> 2000)	4 (5)	Specialists and population in everyday life
	Acute inhalation danger in saturating the concentration of vapor (C 20)	C 20 - clinic	2	For specialists with PPE (respirators, protective glasses, rubber gloves)
		C 20 ≥ Lim ac	3	For specialists with PPE (respirators, protective glasses, rubber gloves)
		C 20 <Lim ac	4	Specialists and population in everyday life
	Acute irritating / corroding effect on skin, scores (erythema, edema)	> 4 (≥ 2.3)	1-2 (1-2)	For specialists with PPE (respirators, rubber gloves)
		2.1 - 4.0 (≥ 1.5 - <2.3)	3 (3)	Specialists and population in life with the use of PPE (latex gloves)
		0 - 2.0 (<1.5)	4 (not classified)	Specialists and population in everyday life
1.1.3. Means for disinfection of ventilation systems and air conditioners (treatment methods: wiping, irrigation)				
1.1.3.1. Tool in the form of aqueous solutions, aerosols actions	Acute toxicity with introduction into the stomach (DL 50 , mg / kg)	> 5000 (> 2000)	4 (5)	For specialists with PPE (respirators, protective glasses, rubber gloves)
	Acute toxicity with application to the skin (DL 50 , mg / kg)	> 2000 (> 2000)	4 (5)	For specialists with PPE (respirators, protective glasses, rubber gloves)
	Acute inhalation danger in saturating the concentration of vapor (C 20)	C 20 <Lim ac	4	To specialists

Appointment facilities	Subjects indicators	Standards Value Indicator	Classification judgment *	Allowed application of
	Skin Resorptive action (21/28 days)	effect lack of effect	It is not classified to specialists using PPE (rubber gloves)	
	Sensitizing effect (skin / respiratory)	lack of effect (no effect)	4 (not classified)	Specialists and population in everyday life
	Acute irritating / corroding effect on skin, scores (erythema, edema)	0 - 2.0 (<1.5)	4 (not classified)	Specialists and population in everyday life
	Acute irritating action on the eyes, scores	0-3	4-5	To specialists
	Inhalation hazard in application mode: Acute toxic zone actions	more than 10	4	Specialists and population in everyday life
1.1.4. Means for disinfection of transport: ground, railway and underground, water, air				
1.1.4.1. Disinfectants means in the form of concentrate, liquid, powder, granules, tablets, gel etc.	Acute toxicity with introduction into the stomach (DL_{50}, mg / kg)	> 150 (> 50 - ≤ 2000)	3-4 (3-4)	For specialists with PPE (respirators, protective glasses, rubber gloves)
		> 5000 (> 2000)	4 (5)	Specialists and population in everyday life
	Acute toxicity with application to the skin (DL_{50}, mg / kg)	> 500 (> 200 - ≤ 2000)	3-4 (3-4)	For specialists with PPE (respirators, protective glasses, rubber gloves)
		> 2000 (> 2000)	4 (5)	Specialists and population in everyday life
	Acute inhalation danger in saturating the concentration of vapor (C_{20})	$C_{20} = Lim_{ac}$	3	For specialists with PPE (safety goggles, latex gloves)
		$C_{20} < Lim_{ac}$	4	To specialists

Appointment facilities	Subjects indicators	Standards Value Indicator	Classification judgment *	Allowed application of
	Acute irritating / corroding effect on skin, scores (erythema, edema)	2.1 - 4.0 (≥ 1.5 - <2.3)	3 (3)	For specialists with PPE (safety goggles, latex gloves)
		0 - 2.0 (<1.5)	4 (not classified)	Specialists and population in everyday life
	Acute irritating action on the eyes, scores	0-3	4-5	To specialists
	Skin Resorptive Action (21/28 days)	effect lack of effect	not classified	For specialists with PPE (rubber gloves)
	Sensitizing effect (skin / respiratory)	moderate / weak (moderate / low)	3A / 3B (1B)	For specialists with PPE (rubber gloves)
		lack of effect (no effect)	4 (not classified)	Specialists and population in everyday life
	Conformity assessment content solution (C) in the air hygienic standards (conducted with	$C / MPC_{(OBUV)\ r.z.} > 1$	3-4	For specialists with PPE (respirators, protective glasses, rubber gloves)
		$C / MPC_{(OBUV)\ r.z.} \leq 1$		To specialists
		$C / MAC_{(OBUV)\ as.} \leq 1$		Specialists and population in

	necessity)			everyday life
1.1.5. Means for disinfection at public catering and trade facilities				
1.1.5.1. Disinfectants means in the form of concentrate, liquid, powder, granules, tablets, gel etc.	Acute toxicity with introduction into the stomach (DL_{50}, mg / kg)	> 150 (> 50 - ≤ 2000)	3-4 (3-4)	For specialists with PPE (respirators, protective glasses, rubber gloves)
		> 5000 (> 2000)	4 (5)	Specialists and population i everyday life
	Acute toxicity with application to the skin (DL_{50}, mg / kg)	> 500 (> 200 - ≤ 2000)	3-4 (3-4)	For specialists with PPE (respirators, protective glasses, rubber gloves)

Appointment facilities	Subjects indicators	Standards Value Indicator	Classification judgment *	Allowed application of
		> 2000 (> 2000)	4 (5)	Specialists and population i everyday life
	Acute inhalation danger in saturating the concentration of vapor (C_{20})	$C_{20} \geq Lim_{ac}$	3	For specialists with PPE (respirators, protective glasses, rubber gloves)
		$C_{20} < Lim_{ac}$	4	Specialists in the presence o of people
	Acute irritating / corroding effect on skin, scores (erythema, edema)	2.1-6.0 (≥ 1.5)	2-3 (2-3)	For specialists with PPE (safety goggles, latex gloves)
		0 - 2.0 (<1.5)	4 (not classified)	Specialists in the presence o of people
	Acute irritating action on the eyes, scores	> 4	2-3	For specialists with PPE (safety goggles, latex gloves)
		0-3	4-5	Specialists in the presence o of people
	Sensitizing effect (skin / respiratory)	moderate / weak (moderate / low)	3A / 3B (1B)	For specialists with PPE (rubber gloves)
		lack of effect (no effect)	4 (not classified)	Specialists in the presence o of people
1.1.5.2. Working solutions disinfecting means	Acute irritating / corroding effect on skin, scores (erythema, edema)	2.1 - 4.0 (≥ 1.5 - <2.3)	3 (3)	For specialists with PPE (rubber gloves)
		0 -2.0 (<1.5)	4 (not classified)	Specialists in the presence o of people
	Irritant effect on skin during repeated applications (0.5-1 months)	moderate / weak	It is not classified to specialists using PPE (rubber gloves)	
		lack of effect		To specialists

Appointment facilities	Subjects indicators	Standards Value Indicator	Classification judgment *	Allowed application of
	Acute irritating action on the eyes, scores	4-6	3	For specialists with PPE (rubber gloves)
		0-3	4-5	To specialists

	Skin Resorptive action (21/28 days)	effect	It is not classified to specialists using	PPE (rubber gloves)
		lack of effect		To specialists
	Inhalation hazard in modes of application:	less than 1	1	According to the epidemo-sheets with PPE application
	Acute toxic zone actions:	1-3	2	(gas mask / respirators, safety glasses, rubber gloves) For specialists with PPE (respirators, protective glasses, rubber gloves) in the absence of people
		3.1-10	3	Specialists in the absence of people
		more than 10	4	To specialists
	Sensitizing effect (skin / respiratory)	moderate / weak (moderate / low)	3A / 3B (1B)	For specialists with PPE (rubber gloves)
		lack of effect (no effect)	4 (not classified)	Specialists in the presence of people
	Safety assessment residual amounts of DS (by choice):			
	- cytotoxicity: cell culture (CCL)	degree CTD - (not more than 1)	not classified	No limits
	bull spermatozoa	toxicity index 70-120%		No limits

Appointment facilities	Subjects indicators	Standards Value Indicator	Classification judgment *	Allowed application of
	Flushing control (chemical analytical method Residual quantities active substance)	absence of residues	Not classified Unlimited	
	Conformity assessment content solution (C) in the air hygienic standards (conducted with necessity)	C / MPC (OBUV) r.z. > 1	2-4	Specialists with use of PPE (respirators, protective glasses, rubber gloves) in general cleaning Specialists in the absence of people Specialists in the presence of people
		C / MPC (OBUV) r.z. ≤1	2-4	
		C / MAC (OBUV) a.n.m ≤1	3-4	
1.1.6. Disinfectants for rapid action in emergency situations				
1.1.6.1. Disinfectants means in the form of concentrate, liquid, powder. tablets	Acute toxicity with introduction into the stomach (DL 50 , mg / kg)	> 15 (> 5-≤ 2000)	2-4 (2-4)	For specialists with PPE (respirators, protective glasses, rubber gloves) Specialists and population in everyday life
		> 5000 (> 2000)	4 (5)	
	Acute toxicity with application to the skin (DL 50 , mg / kg)	> 100 (> 50-≤ 2000)	2-4 (2-4)	For specialists with PPE (safety goggles, latex gloves) Specialists and population in everyday life
		> 2000 (> 2000)	4 (5)	
	Acute inhalation danger in saturating the concentration of vapor (C 20)	With 20- gable C 20 - clinic	1-2	Specialists in the epidemic. readings using PPE (gas mask / respirators,

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Appointment facilities	Subjects indicators	Standards Value Indicator	Classification judgment *	Allowed application of
		$C_{20} \geq Lim_{ac}$	3	safety glasses, rubber gloves) For specialists with
		$C_{20} < Lim_{ac}$	4	PPE (respirators, protective glasses, rubber gloves) To specialists
	Acute irritating / corroding effect on skin, scores (erythema, edema)	> 6 (necrosis)	1 (1)	For specialists with PPE (respirators, protective glasses, rubber gloves)
		2.1-6.0 (≥ 1.5)	2-3 (2-3)	For specialists with PPE (safety goggles, latex gloves)
		0 - 2.0 (<1.5)	4 (not classified)	Specialists and population i everyday life
	Acute irritating action on the eyes, scores	> 4	1-3	For specialists with PPE (respirators, protective glasses, rubber gloves)
		0-3	4	To specialists
	Sensitizing effect (skin / respiratory)	moderate / weak (moderate / low)	3A / 3B (1B)	For specialists with PPE (rubber gloves)
		lack of effect (no effect)	4 (not classified)	Specialists and population i everyday life
1.1.6.2. Working solutions disinfecting means	Inhalation hazard in application mode: Acute toxic zone actions	less than 1	1	According to the epidemo-sheets with PPE application (gas mask / respirators, safety glasses, rubber gloves)
		1-3	2	For specialists with PPE (respirators, protective

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Appointment facilities	Subjects indicators	Standards Value Indicator	Classification judgment *	Allowed application of
				glasses, rubber gloves) in the absence of people
		3.1-10	3	Specialists in the absence of people
		more than 10	4	To specialists
1.1.7. Means for disinfection and pre-sterilization cleaning of medical devices, including endoscopes, dental instruments and prints				
1.1.7.1. Means in the form of - concentrate, liquid, powder, granules, etc.	Acute toxicity with introduction into the abdominal cavity (DL_{50}, mg / kg)	not less than 11	2-6	To specialists
	Acute toxicity with introduction into the stomach (DL_{50}, mg / kg)	> 150 (> 50 - ≤ 2000)	3-4 (3-4)	For specialists with PPE (respirators, protective glasses, rubber gloves)
		> 5000 (> 2000)	4 (5)	Specialists and population i everyday life

	Acute toxicity with application to the skin (DL 50 , mg / kg)	> 500 (> 200 - ≤ 2000)	3-4 (3-4)	For specialists with PPE (respirators, protective glasses, rubber gloves)
		> 2000 (> 2000)	4 (5)	Specialists and population in everyday life
	Acute inhalation danger in saturating the concentration of vapor (C 20)	C 20 ≥ Lim ac	3	For specialists with PPE (respirators, protective glasses, rubber gloves)
		C 20 <Lim ac	4	For specialists with PPE (rubber gloves)
	Acute irritating / corroding effect on skin, scores (erythema, edema)	> 4 (≥ 2.3)	1-2 (1-2)	For specialists with PPE (rubber gloves)
		0 - 2.0 (<1.5)	4 (not classified)	Specialists and population in everyday life

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Appointment facilities	Subjects indicators	Standards Value Indicator	Classification judgment *	Allowed application of
	Acute irritating action on the eyes, scores	> 4 <10	2-3	For specialists with PPE (safety goggles, latex gloves)
		0-3	4	To specialists
	Sensitizing effect (skin / respiratory)	moderate / weak (moderate / low)	3A / 3B (1B)	For specialists with PPE (rubber gloves)
		lack of effect (no effect)	4 (not classified)	Specialists and population in everyday life
1.1.7.2. Working solutions facilities	Acute irritating / corroding effect on skin, scores (erythema, edema)	2.1 - 4.0 (≥ 1.5 - <2.3)	3 (3)	For specialists with PPE (safety goggles, latex gloves)
		0 - 2.0 (<1.5)	4 (not classified)	Specialists and population in everyday life
	Irritant effect on skin during repeated applications (0.5-1 months)	moderate / weak	It is not classified to specialists using PPE (rubber gloves)	
		lack of effect		To specialists
	Safety assessment residual amounts of DS (by choice):			
	- cytotoxicity: cell culture (CCL)	degree CTD - (not more than 1)	not classified	No limits
	bull spermatozoa	toxicity index		No limits
	- hemolysis of erythrocytes	70-120% not more than 2%		No limits
	- pyrogenicity	lack of effect		No limits

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Appointment facilities	Subjects indicators	Standards Value Indicator	Classification judgment *	Allowed application of

1.1.8. Means for disinfecting the surfaces of process equipment and premises at enterprises in various branches of the food industry

1.1.8.1. Disinfectant means in the form of - concentrate, liquid, powder, granules, aerosols, etc.	Acute toxicity with introduction into the stomach (DL $_{50}$, mg / kg)	> 150 (> 50 - ≤ 2000)	3-4 (3-4)	For specialists with PPE (respirators, protective glasses, rubber gloves)
		> 5000 (> 2000)	4 (5)	Specialists and population in everyday life
	Acute toxicity with application to the skin (DL $_{50}$, mg / kg)	> 500 (> 200 - ≤ 2000)	3-4 (3-4)	For specialists with PPE (respirators, protective glasses, rubber gloves)
		> 2000 (> 2000)	4 (5)	Specialists and population in everyday life
	Acute inhalation danger in saturating the concentration of vapor (C_{20})	C_{20} - clinic	2	For specialists with PPE (gas mask / respirators safety glasses, rubber gloves)
		$C_{20} \geq Lim_{ac}$	3	For specialists with PPE (respirators, protective glasses, rubber gloves)
		$C_{20} < Lim_{ac}$	4	To specialists
	Acute irritating / corroding effect on skin, scores (erythema, edema)	> 6 (necrosis)	1 (1)	For specialists with PPE (respirators, protective glasses, rubber gloves)
		2.1-6.0 (≥ 1.5)	2-3 (2-3)	For specialists with PPE (safety goggles, latex gloves)
		0 - 2.0 (<1.5)	4 (not classified)	Specialists and population in everyday life

Appointment facilities	Subjects indicators	Standards Value Indicator	Classification judgment *	Allowed application of
	Acute irritating action on the eyes, scores	> 4	1-3	For specialists with PPE (respirators, protective glasses, rubber gloves)
		0-3	4	To specialists
	Cumulative effect (by classification of Bear LI)	3.1-5	moderate	To specialists
		> 5	weak	To specialists
	Sensitizing effect (skin / respiratory)	moderate / weak (moderate / low)	3A / 3B (1B)	For specialists with PPE (rubber gloves)
		lack of effect (no effect)	4 (not classified)	Specialists and population in everyday life
1.1.8.2. Working solutions disinfectant facilities	Acute irritating / corroding effect on skin, scores (erythema, edema)	2.1 - 4.0 (≥ 1.5 - <2.3)	3 (3)	For specialists with PPE (safety goggles, latex gloves)
		0 - 2.0 (<1.5)	4 (not classified)	Specialists and population in everyday life
	Irritant effect on skin during repeated applications (0.5-1 months)	moderate / weak	It is not classified to specialists using	PPE (rubber gloves)
		lack of effect		To specialists
	Safety assessment residual amounts of DS (by choice):			
	- cytotoxicity: cell culture (CCL)	degree CTD - (not more than 1)	not classified	No limits
	- Bull spermatozoa	toxicity index		
	Flushing control (according to the results studies of the chemical-	70-120% absence of residues	Not classified Unlimited	

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Appointment facilities	Subjects indicators	Standards: Value Indicator	Standards: Classification judgment *	Allowed application of
	residual amounts of DW)			
1.1.9. Antimicrobial materials				
	Conformity assessment content solution (C) in the air hygienic standards (conducted with necessity)	C / MPC (OBUV) r.z. > 1	2-4	Specialists with use of PPE (respirators, protective glasses, rubber gloves)
		C / MPC (OBUV) r.z. ≤1	2-4	To specialists
		C / MAC (OBUV) as. ≤1	3-4	Specialists in the presence o people and people in every
1.1.9.2. Disinfectants means for giving tissues of antimicrobial properties	Sensitizing act (skin / respiratory)	lack of effect (no effect)	4 (not classified)	Staff, patients and population in everyday life
	Toxicity analysis for bull spermatozoa (stretch from the fabric)	toxicity index 70-120%		Staff, patients and population in everyday life
	Skin resorptive and irritating effect on skin remedies and tissues (14/28 days)	Absence of effects	It is not classified as Personnel,	Patients and population in everyday life

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1.2. Sterilizing agents

Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Normative indicators: Classification assessment of	Allowed application of
1.2.1. Sterilizing means in the form of concentrate, liquid, powder etc.	Acute toxicity with introduction into the stomach (DL 50 , mg / kg)	> 15 (> 5-≤ 2000)	2-4 (2-4)	Specialists with use of PPE (respirators, protective glasses, rubber gloves)
		> 5000 (> 2000)	4 (5)	To specialists
	Acute toxicity with application to the skin (DL 50 , mg / kg)	> 100 (> 50-≤ 2000)	2-4 (2-4)	Specialists with use of PPE (goggles, rubber gloves)
		> 2000 (> 2000)	4 5	To specialists
	Acute toxicity with			

	introduction into the abdominal cavity (DL $_{50}$, mg / kg)	not less than 11	2-6	To specialists
	Acute inhalation danger in saturating the concentration of vapor (C $_{20}$)	C $_{20}$ - clinic	2	Specialists with use of PPE (gas mask / respirators, safety glasses, rubber gloves)
		C $_{20}$ ≥ Lim $_{ac}$	3	Specialists with use of PPE (respirators, protective glasses, rubber gloves)
		C $_{20}$ <Lim $_{ac}$	4	To specialists
	Acute irritating / corroding effect on skin, scores (erythema, edema)	> 6 (necrosis)	1 (1)	Specialists with use of PPE (respirators, protective glasses, rubber gloves)
		2.1-6.0	2-3	

Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Classification assessment of	Allowed application of
		(≥ 1.5)	(2-3)	Specialists with use of PPE (goggles, rubber gloves)
		0 - 2.0 (<1.5)	4 (not classified)	Specialists and population at home
	Sensitizing act (skin / respiratory)	moderate / weak (moderate / low)	3A / 3B (1B)	Specialists with use of PPE (latex gloves)
		lack of effect (lack of effect)	4 (not classified)	Specialists and population at home
1.2.2. Extracts (extracts or rinses) from medical products	Safety assessment residual amounts of DS (by choice):			
	- cytotoxicity: cell culture (CCL)	degree CTD - (not more than 1)	not classified	No limits
	bull spermatozoa	toxicity index		No limits
	- hemolysis of erythrocytes	70-120% not more than 2%		No limits
	- pyrogenicity	lack of effect		No limits
1.2.3. Working solutions sterilizing agents	Acute irritating / corroding effect on skin, scores (erythema, edema)	2.1 - 4.0 (≥ 1.5 - <2.3)	3 (3)	Specialists with use of PPE (goggles, rubber gloves)
		0 - 2.0 (<1.5)	4 (not classified)	Specialists and population at home

Appointment facilities	Subjects indicators	Normative indicators: Value	Classification	Allowed application of

	indicator	assessment of	
Irritant effect on skin during repeated applications (0.5-1 months)	moderate / weak	not classified	
	lack of effect		use of PPE (latex gloves) To specialists

1.3. Means for disinfection drinking water of decentralized water supply

Appointment facilities	Investigated indicators	Normative indicators: Value indicator	Normative indicators: Classification assessment of	Allowed application of
1.3.1. Means for disinfection of drinking water water of non-centralized water supply (tablets, powders, solutions, granules).	Acute toxicity when injected into the stomach, (DL_{50}, mg / kg)	> 150 (> 50-≤ 2000)	3-4 (3-4)	Specialists with use of PPE (latex gloves)
		> 5000 (> 2000)	4 (5)	Specialists and populatio at home
	Acute toxicity when applied to skin (DL_{50}, mg / kg).	151 - 2500 (> 200-≤ 1000)	3 (3)	Specialists and populatio in the home with the use (latex gloves)
		> 2500 (> 1000)	4 (4-5)	Specialists and populatio at home
	Acute inhalation hazard of workers solutions in maximum concentration (at saturating concentrations at 20 to C (C_{20})	With $_{20}$ = Lim $_{ac}$	3	Specialists with application of PPE (safety goggles, latex gloves)
		With $_{20}$ > Lim $_{ac}$	4	Specialists and populatio at home

Appointment facilities	Investigated indicators	Normative indicators: Value indicator	Normative indicators: Classification assessment of	Allowed application of
	Irritant effect on the skin of workers solutions in maximum concentration (again, 10 applications), points	2.1 - 4.0 (≥2,3 - <4)	3 (2)	Specialists with use of PPE (latex gloves)
		2.1 - 4.0 (≥1.5 - <2.3)	3 (3)	Specialists and populatio in the home with the use (latex gloves)
		0 - 2.0 (<1.5)	4 not classified	Specialists and the public at home
	Irritant effect of the drug on mucous membranes of the eyes of workers solutions in various concentrations (once), points	1-6	3-4	Specialists with use of PPE (goggles, rubber gloves)
		1-3	4	Specialists and populatio in the home with the use (goggles, rubber gloves)
		0	5	Specialists and populatio at home
	Skin / respiratory sensitizing effect of workers solutions in maximum concentration (according to indications)	lack of effect	not is classified	Specialists and populatio at home
	Remote effects: embryotoxic, mutagenic, carcinogenic, embryotoxic,	lack of	not	Production and

	teratogenic (taking into account the data literature, databases on DV and related components .)	effect	is classified	application of the facility

Appointment facilities	Investigated indicators	Normative indicators: Value indicator	Normative indicators: Classification assessment of	Allowed application of
1.3.2. Drinking water, processed disinfectant means in the mode applications	Tests of disinfected natural water (lake, pond, well, river, etc.) in the regime applications			
	The ratio of the minimum effective disinfection concentration (МЭОК) and ПДКв. on DV (МЭОК / ПДКК.)	> 10		Occasional application of the agent o ban
		> 5 - 10	not is classified	Permission is granted to u funds within 10-15 days.
		> 1 - 5		Permission is granted to u funds within 30 days
		≤1		Permission is granted to u unlimited means duration
	Definition of the subacute threshold disinfected decentralized water by time the onset of the effect, Lim subac (days) 1)	1-3		Episodic application water or ban
		thirty		The use of water for 10-15 days
		90		The use of water for months
	Organoleptic properties disinfected water in application (smell, taste), scores	≤ admissible meanings	not classified	Permission is granted to u facilities
	Sanitary and chemical indicators water safety	≤ admissible meanings	not classified	Permission is granted to u facilities
	The concentration of DV in disinfected water	≤ MAC	not is classified	Permission is granted to u facilities
	Concentration of products transformation of DV and products transformation, formed under the	≤ MAC	not is classified	Permission is granted to u facilities

Appointment facilities	Investigated indicators	Normative indicators: Value indicator	Normative indicators: Classification assessment of	Allowed application of
	the influence of DV, in water			
	Concentration of harmful impurities means of disinfection in water	≤ 0.5DPKv	not is classified	Permission is granted to u facilities

1) Determination of the zone of subacute biocide effect of the means of calculation by formula

$$Z_{subac\ .bioc\ .eff} = \mathrm{Lim}_{subac}\ (\ by\ \ limiting\ \ \ \ him\ \ effect\ \ \ \ )$$

Daily norm expenditure

1.4. Means for disinfecting the water of swimming pools and water parks

Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Normative indicators: Classification assessment of	Allowed application
1.4.1. Tools for disinfection of water swimming pools and water parks (tablets, powders, liquids, granules).	Acute toxicity with introduction into the stomach, (DL_{50}, mg / kg).	151 - 5000	3	Specialists with use of PPE (latex gloves)
		(> 50 - ≤300)	(3)	
		> 2500	4	Specialists and population everyday life
		(> 300)	(4-5)	
	Acute toxicity with applying to the skin, (DL_{50} mg / kg).	> 500	3-4	Specialists and population life with the use of PPE (latex gloves)
		(> 200 - ≤2000)	(3-4)	
		> 2000	4	Specialists and population everyday life
		(> 2000)	(5)	

Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Normative indicators: Classification assessment of	Allowed application
	Inhalation hazard funds in saturating the concentration of vapor (C_{20}).	With $_{20}$ > Lim_{ac}	2-3	Specialists with use of PPE (respirators)
		With $_{20}$ <Lim_{ac}	4	Specialists and population everyday life
	Irritant effect means for the skin of *workers solutions in the maximum concentration* (15 applications), points	2.1 - 4.0	3	Specialists with use of PPE (latex gloves)
		(≥2,3 - <4)	(2)	
		2.1 - 4.0	3	Specialists and population in the home with the use of
		(≥1.5 - <2.3)	(3)	(latex gloves)
		0 - 2.0	4	Specialists and population everyday life
		(<1.5)	(not classified)	
	Irritant effect on mucous membranes of the eyes *working solutions in maximum concentration* (once), points	4-6	3	Specialists with use of PPE (protective glasses)
		1-3	4	Specialists and population use of PPE (protective glasses)
		0	5	Specialists and population everyday life
	Sensitizing act (skin / respiratory) *working solutions in maximum concentration* (according to indications)	lack of effect (no effect)	4 (not classified)	Specialists and population everyday life

Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Normative indicators: Classification assessment of	Allowed application
	Remote effects: embryotoxic, mutagenic, carcinogenic, embryotoxic, teratogenic (including data of literature, databases data on LW and accompanying components .)	no effect is not classified		Production and application of the facility
	Residual disinfectant (bacteriostatic) act	Effect Duration effect - ≥1 days with permanganate water oxidation 5 mgO 2 / L	not classified	Permission is granted to us facilities
	The ratio of the minimum effective disinfecting concentration (МЭОК) and MAC of the bass. DV (МЭОК / ПДК басс[1)])	> 1		The use of a permissible
		≤1	not classified	Permission is granted to us facilities
	Testing of disinfected water in a swimming pool or water park in the application mode			
1.4.2. Water swimming pool or water park, processed disinfectant means in the mode applications	Determination of threshold subacute action (28 days) disinfected water integrated impact (intragastric,	= 1-5 N		Prohibition of use facilities
		10 N		Specialists and adults population in everyday life
		> 10 N		Specialists and population i everyday life

Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Normative indicators: Classification assessment of	Allowed application
	inhalation, skin-resorptive), depending from consumption rates (N), Lim subac			
	Concentration of DV in water swimming pool or water park	≤ MAC limit for bass.	not classified	Permission is granted to use facilities
	Concentration of products transformation of water in water	≤ MAC limit for bass.	not classified	Permission is granted to use facilities
	Concentration of products transformation, formed under the influence of DV in water	≤ MPCs.	not classified	Permission is granted to use facilities
	Concentration of harmful impurities means disinfection in water	≤ 0,5PDC.	not classified	Permission is granted to use facilities
	The concentration of DV in the air in the zone of breathing swimmers	≤ MPC.a.	not classified	Permission is granted to use facilities
	Organoleptic properties disinfected water in mode of application (odor, aftertaste, turbidity,	≤ allowable meanings	not classified	Permission is granted to use facilities

	chromaticity)			
	Organoleptic properties disinfected water in application mode (foaming)	lack of	not classified	Permission is granted to use facilities

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Appointment facilities	Subjects indicators	Normative indicators		Allowed application
		Value indicator	Classification assessment of	
	Sanitary and chemical safety indicators water (pH, permanganate oxidizability)	≤ allowable meanings	not classified	Permission is granted to use facilities

1) is calculated by the formula:

$$\text{MAC of the}_{\text{bass.}} = \frac{DD_{ent}\ (mg/kg) \cdot M\ (kg)}{V\ (l/h) \cdot t\ (h)},\ mg/l$$

DD_{ent} - the permissible dose that enters the body when swallowing the swimming pool water, mg / kg;
M - average weight of swimmers taking into account children (45 kg);
V - volume of the swallowed liquid (0.1 l / h);
t - duration of the bathing session (3 hours).

$$DD_{ent} = DDD - DD_{skin} - DD_{ing}\text{, where}$$

DDD is the permissible daily dose;
DD_{skin} - the permissible dose, which enters the body transcutaneously during bathing, mg / kg;
DD_{ing} - the permissible dose, entering the body upon inhalation of the drug, evaporating from the water, mg / kg.

Or

$$\text{MAC of the}_{\text{bass.}} = \frac{DDD\ (mg/kg) \cdot M\ (kg)}{V\ (l/h) \cdot t\ (h)},\ mg/l\text{, where}$$

DDD is the permissible daily dose;
M - average weight of swimmers taking into account children (45 kg);
V - volume of the swallowed liquid (0.1 l / h);
t - duration of the bathing session (3 hours).

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1.5. Means for water disinfection of centralized systems of domestic and drinking water supply, in t.ch. in hot water systems

Appointment facilities	Subjects indicators	Normative indicators		Allowed application
		Value indicator	Classification assessment of	
1.5.1. Means for disinfection of water centralized systems household and drinking	Acute toxicity with introduction into the stomach, (DL_{50}, mg / kg).	> 150 (> 50 - ≤2000)	3-4 (3-4)	Specialists with use of PPE (latex gloves) Specialists and populatio

water supply, incl. at systems of hot water supply (gaseous, liquid, tablets, powders, granules).		(≥ 5000)	4	at home
		(> 2000)	(5)	Specialists and populatio
	Acute toxicity with applying to the skin, (DL 50 mg / kg).	> 500 (> 200 - ≤2000)	3-4	Specialists with use of PPE (latex gloves)
		> 2000 (> 2000)	4 (5)	Specialists and populatio at home
	Inhalation hazard funds in saturating the concentration of vapor (C 20).	With 20 > Lim ac	2-3	Specialists with use of PPE (respirators, rubber gloves)
		With 20 <Lim ac	4	Specialists and populatio at home
	Irritant effect means for the skin of workers solutions in the maximum concentration (15 applications), points	2.1 - 4.0 (≥1.5 - <4)	3 (2-3)	Specialists with use of PPE (latex gloves)
		0 - 2.0 (<1.5)	4 (not classified)	Specialists and populatio everyday life

Appointment facilities	Subjects indicators	Normative indicators		Allowed application
		Value indicator	Classification assessment of	
	Irritant effect on mucous membranes of the eyes working solutions in maximum concentration (once), points	1-6	3-4	Specialists with use of PPE (goggles, rubber gloves) To specialists
		0	5	
	Sensitizing action of working solutions in the maximum concentrations	lack of effect (no effect)	4 (not classified)	Production and application of the facility
	Remote effects: embryotoxic, mutagenic, carcinogenic, embryotoxic, teratogenic (including data of literature, databases data on LW and related components)	lack of effect	not classified	Production and application of the facility
	Residual disinfectant (bacteriostatic) act	Effect Duration effect - ≥1 days with permanganate water oxidation 5 mgO 2 / L	not classified	Permission is granted to facilities
	The ratio of the minimum effective disinfecting concentration (MƎOK) and MPCs. DV (MEOK / PDKv.)	> 1		The use of a permissible
		≤1	not classified	Permission is granted to facilities

Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Normative indicators: Classification assessment of	Allowed application
1.5.2. Water centralized systems household and drinking water supply, incl. at systems of hot water supply, processed disinfectant means in the mode applications	Tests of disinfected water of centralized systems of domestic and drinking water supply, incl. at systems of hot water supply, in the mode of application			
	Concentration of LW in disinfected water	≤ MAC	not classified	Permission is granted to us facilities
	Concentration of products transformation of DW and products of transformation, formed under the influence of DV, in water	≤ MAC	not classified	Permission is granted to us facilities
	Concentration of harmful impurities means disinfection in water	≤ 0.5DPKv	not classified	Permission is granted to us facilities
	Organoleptic properties disinfected water in mode of application (odor, smack, turbidity, color)	≤ allowable meanings	not classified	Permission is granted to us facilities
	Sanitary and chemical safety indicators water (pH, permanganate oxidizability)	≤ allowable meanings	not classified	Permission is granted to us facilities

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1.6. Means for disinfection in technical water supply systems of enterprises

Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Normative indicators: Classification assessment of	Allowed application
1.6.1. Means for disinfection of water in technical water supply enterprises (tablets, powders, liquid, granules).	Acute toxicity with introduction into the stomach, (DL_{50}, mg / kg).	> 150	3-4	Specialists with use of PPE (latex gloves)
		(> 50 - ≤2000)	(3-4)	
		> 5000	4	Specialists and population everyday life
		(> 2000)	(5)	
	Acute toxicity with applying to the skin, (DL_{50} mg / kg).	> 500	3-4	Specialists with use of PPE (latex gloves)
		(> 200-≤ 2000)	(3-4)	
		> 2000	4	Specialists and population everyday life
		(> 2000)	(5)	
	Inhalation hazard funds in saturating the concentration of vapor (C_{20}).	With $_{20}$ > Lim $_{ac}$	2-3	Specialists with use of PPE (respirators, rubber gloves)
		With $_{20}$ <Lim $_{ac}$	4	Specialists and population everyday life
	Irritant effect	2.1 - 4.0	3	Specialists with

means for the skin of *workers solutions in the maximum concentration* (15 applications), points	0 - 2.0	4	use of PPE (latex gloves) Specialists and population everyday life
Irritant effect on mucous membranes of the eyes *working solutions in maximum concentration* (once), points	1-6	3-4	Specialists with use of PPE (goggles, rubber gloves)
	0	5	To specialists

Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Normative indicators: Classification assessment of	Allowed application
	Sensitizing act (skin / respiratory) *working solutions in maximum concentration*	lack of effect (no effect)	4 (not classified)	Production and application of the facility
	Remote effects: embryotoxic, mutagenic, carcinogenic, embryotoxic, teratogenic (including data of literature, databases data on LW and related components)	lack of effect	not classified	Production and application of the facility
	Residual disinfectant (bacteriostatic) act	Effect Duration effect - ≥1 days with permanganate water oxidation 5 mgO 2 / l	not classified	Permission is granted to us facilities

1.7. Disinsection means

Assignment of funds	Investigated indicators	Normative indicators: Value indicator	Normative indicators: Classification assessment of	Allowed application
1.7.1. Aerosol cans Zone of acute biocide	effect	<10	1	Specialists with use of PPE in

Acid biocidal zone effect for alcohol-containing products decreases by an order of magnitude			According to current legislation and working conditions in extreme situations.
	10 - 30	2	Specialists with use of PPE (respirators, protective glasses, rubber gloves, overalls, etc.)
	31 - 100	3	Specialists and population in everyday life with regulated terms of use (ventilation, consumption preparation, wet cleaning etc.)
	> 100	4	Specialists of the populatio
Zone of subacute biocide effect	<1	1	Prohibited for use in pest control
	1-5	2	Specialists for processing production premises with regulated

	5.1-10	3	terms of use for the exception of children's life.
	> 10	4	Specialists and population in everyday life with regulated terms of use (drug consumption, airing, cleaning premises) for processing industrial and residential premises
			Specialists and population at home
Acute toxicity with insertion into the stomach without propellant, (DL 50 , mg / kg)	151 - 5000 (> 50 - ≤ 300)	3 (3)	Specialists with use of PPE (latex gloves)
	> 5000 (> 300)	4 (4-5)	Specialists and population in everyday life
Acute irritating / corroding effect on skin, scores (erythema, edema)	2.1 - 4.0 (≥ 1.5 - <2.3)	3 (3)	Specialists and population in the home with the use o (latex gloves)
	0 - 2.0 (<1.5)	4 (not classified)	Specialists and population at home
Acute irritating action on the eyes, scores	<10	2-3	Specialists with use of PPE (protective glasses).
	0-3	4-5	Specialists and population at home
Sensitizing effect (skin / respiratory)	moderate / weak (moderate / low)	3A / 3B (1B)	Specialists with use of PPE (respirators, protective glasses, rubber gloves,

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		lack of effect (lack of effect)	4 (not classified)	overalls, etc.) Specialists and population at home
	Conformity assessment content solution (C) in the air hygienic standards (conducted with necessity)	C / MPC (OBUV) r.z. > 1	2-4	Specialists with use of PPE (respirators, protective glasses, rubber gloves) Specialists in the absence of people Specialists and population at home
		C / MPC (OBUV) r.z. ≤1	2-4	
		C / MAC (OBUV) as. ≤1	3-4	
1.7.2. Pyrotechnic and fumigating agents (drafts, tablets, candles, liquid, etc.)	Acid biocidal zone effect	<10	1	Specialists with use of PPE (gas masks / respirators, sealed goggles, latex gloves, overalls, etc.) Specialists with use of PPE (respirators, hermetic glasses, rubber gloves, overalls, etc.) Specialists and population in everyday life with regulated terms of use (ventilation, wet cleaning, consumption of t Specialists and population at home
		10 - 30	2	
		31 - 100	3	
		> 100	4	

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	Zone of subacute biocide effect	less than 1	1	Prohibited for use in pest control Specialists for processing production premises with regulated terms of use for the exception of children's way of life Specialists and population in the home for processing and production premises with regulated terms of use (cleaning, airing, consumption of the drug) Specialists and population at home Specialists with
		1-5	2	
		5.1-10	3	
		> 10	4	
	Acute toxicity with		3	

	introduction into the stomach, (DL 50 , mg / kg)	151 - 5000 (> 50-≤ 300) > 5000 (> 300)	(3) 4 (4-5)	use of PPE (latex gloves) Specialists and population at home
	Acute irritating action on the eyes, scores	<10 0-3	2-3 4-5	Specialists with use of PPE (protective glasses) Specialists and population at home

	Sensitizing effect (skin / respiratory)	moderate / weak (moderate / low) lack of effect (lack of effect)	3A / 3B (1B) 4 (not classified)	Specialists with use of PPE (respirators, hermetic glasses, rubber gloves, overalls, etc.) Specialists and population at home
	Conformity assessment content solution (C) in the air hygienic standards (conducted with necessity)	C / MPC (OBUV) r.z. > 1 C / MPC (OBUV) r.z. ≤1 C / MAC (OBUV) as. ≤1	2-4 2-4 3-4	Specialists with use of PPE (respirators, protective glasses, rubber gloves) Specialists in the absence of people Specialists and population at home
1.7.3. Emulsifying concentrates, wettable powders, microencapsulated concentrates, varnishes, paints, solutions	a) Remedy: Acute toxicity with introduction into the stomach (DL 50 , mg / kg).	151 - 5000 (> 50-≤ 300) > 5000 (> 300)	3 (3) 4 4-5	Specialists with use of PPE (latex gloves) Specialists and population in everyday life
	Acute toxicity with application to the skin (DL 50 , mg / kg).	> 500 (> 200 - ≤ 2000) > 2000 (> 2000)	3-4 (3-4) 4 (5)	Specialists with use of PPE (latex gloves). Specialists and population at home
	Acute inhalation danger in saturating concentrations (C 20)	C 20 - clinic With 20 > Lim ac	2 3-4	Specialists with use of PPE (respirators, protective glasses, rubber gloves) Specialists and population at home
	Acute irritating /	4.1-6.0	2	Specialists with

	corroding effect on skin, scores (erythema, edema)	(≥ 2.3 - <4.0) 2.1 - 4.0 (≥ 1.5 - <2.3) 0 - 2.0 (<1.5)	(2) 3 (3) 4 (not classified)	use of PPE (latex gloves) Specialists and population at home Specialists and population at home

Acute irritating action on the eyes, scores	<10	2-3	Specialists with use of PPE (protective glasses)
	0-3	4-5	Specialists and population at home
Sensitizing effect (skin / respiratory)	moderate / weak (moderate / low)	3A / 3B (1B)	Specialists with use of PPE (latex gloves)
	lack of effect	4	Specialists and population at home
	(lack of effect)	(not classified)	
b) Working emulsions, suspensions, solutions			
Skin Resorptive action (21/28 days)	effect	not classified	use of PPE (latex gloves)
	lack of effect		Specialists and population at home
Irritant effect on skin during repeated applications (0.5-1 months)	moderate / weak	Not classified by specialists and population	in the home with the use o (latex gloves)
	lack of effect		Specialists and population at home

Acute biocidal zone effect	<10	1	Specialists with use of PPE (gas masks / respirators, sealed goggles, latex gloves, overalls, etc.)
	10 - 30	2	Specialists with use of PPE (respirators, hermetic glasses, rubber gloves, overalls, etc.)
	31 - 100	3	Specialists and populatior in everyday life with regulated terms of use (cleaning, airing).
	> 100	4	Specialists and population at home
Zone of subacute biocide effect	<1	1	Prohibited for use in pest control
	1-5	2	Specialists for processing production premises with regulated terms of use for children
	5.1-10	3	institutions, MOs and life Specialists and populatior in the home for processing and production premises with regulated terms of use

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		> 10	4	(cleaning, airing, consumption of the drug) Specialists and populatior at home
	Sensitizing effect (skin / respiratory)	moderate / weak (moderate / low)	3A / 3B (1B)	Specialists with use of PPE (latex gloves, respirators)
		lack of effect (lack of effect)	4 (not classified)	Specialists and population at home
	Conformity assessment content solution (C) in the air hygienic standards (conducted with necessity)	C / MPC (OBUV) r.z. > 1	2-4	Specialists with use of PPE (respirators, protective glasses, rubber gloves)
		C / MPC (OBUV) r.z. ≤1	2-4	Specialists in the absence of people
		C / MAC (OBUV) as. ≤1	3-4	Specialists and population at home
1.7.4. Dusts, pencils, briquettes, baits, ready to use solutions, emulsions, suspensions, tablets, gels	Acute toxicity with introduction into the stomach (DL 50 , mg / kg).	151 - 5000 (> 50 - ≤ 300) > 5000 (> 300)	3 (3) 4 (4-5)	Specialists with use of PPE (latex gloves) Specialists and population at home.
	Acute toxicity with applying to the skin, (DL 50 , mg / kg).	> 500 (> 200 - ≤ 2000) > 2000 (> 2000)	3-4 (3-4) 4 (5)	Specialists with use of PPE (latex gloves). Specialists and population at home
	Acute inhalation danger in saturating concentrations (C 20)	C 20 - clinic	2	Specialists with use of PPE (respirators, protective glasses, rubber gloves)
		With 20 <Lim ac	3-4	Specialists and population

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			at home
	Acid biocidal zone effect	<10	1 Specialists with use of PPE (gas masks / respirators, sealed goggles, latex gloves, overalls, etc.)
		10 - 30	2 Specialists with use of PPE (respirators, hermetic glasses, rubber gloves, overalls, etc.)
		31 - 100	3 Specialists and populatior in everyday life with regulated terms of use (cleaning, airing). Specialists and population
		> 100	4

	Zone of subacute biocide effect	<1	1
		1-5	2
		5.1-10	3
		> 10	4
	Skin Resorptive action (21/28 days)	effect	not classified
		lack of effect	
	Irritant effect on skin during repeated applications (0.5-1 months)	moderate / weak	Not classified by specialists and population
		lack of effect	
	Sensitizing effect (skin / respiratory)	moderate / weak (moderate / low)	3A / 3B (1B)
		lack of effect (lack of effect)	4 (not classified)
	Acute irritating action on the eyes, scores	<10	2-3
		0-3	4-5
	Conformity assessment content solution (C) in the air hygienic standards (conducted with necessity)	C / MPC (OBUV) r.z. > 1	2-4
		C / MPC (OBUV) r.z. ≤1	2-4
		C / MAC (OBUV) as. ≤1	3-4
1.7.5. Insecticidal means for impregnation and	Toxicity analysis for bull spermatozoa	toxicity index 70-120%	Staff, patients and the population in everyday life

at home
Prohibited for use in pest control
Specialists for processing production premises with regulated terms of use for children institutions, MOs and life
Specialists and populatior in the home for processing and production premises with regulated terms of use (cleaning, airing, consumption of the drug)
Specialists and populatior at home
use of PPE (latex gloves)
Specialists and population at home
Specialists and population in the home with the use o (latex gloves)
Specialists and population at home
Specialists with use of PPE (latex gloves, respirators)
Specialists and population at home
Specialists with use of PPE (protective glasses)
Specialists and population at home
Specialists with use of PPE (respirators, protective glasses, rubber gloves)
Specialists in the absence of people
Specialists and population at home
The State Standard of the I 12/29/1999 Appendix A

processing of tissues	(stretch from the fabric) Skin resorptive and irritating effect on skin means and tissues (0.5-1 months)	no effects	not standardized	Staff, patients and population in everyday life

1.8. Repellent means

Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Classification assessment of	Allowed application of
1.8.1. Repellent means for application to skin	Acute toxicity with applying to the skin, (DL 50 mg / kg)	> 2500 (> 2000 ≤ 5000)	4 (5)	1) Working staff
		> 2500 (> 5000)	4 (not classified)	1) Working staff adult and 2) children's population
1.8.1.1. Creams, emulsions, lotions, pencils, bracelets, etc.	Acute toxicity with introduction into the stomach, (DL 50 , mg / kg)	> 150 (> 300 - ≤ 5000)	3-4 (4-5)	1) Working staff
		> 5000 (> 5000)	4 (not classified)	Working staff, adult and 2) child population
	Acute inhalation danger in saturating the concentration of vapor (C 20)	With 20 = Lim ac	3	Working staff and adult population in everyday
		With 20 <Lim ac	4	Working staff, adult and 2) child population
	Irritant effect on	weak		Working staff in

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Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Classification assessment of	Allowed application of
	skin during repeated applications (0.5-1 months)		is not classified as a regulated mode	applications
		lack of effect		Working staff, adult population children
	Acute irritating action on the eyes, scores	4.0-6.0	3	Working staff
		0-2.0	4-5	Working staff, adult and 2) child population
	Skin resorptive and irritating effect on skin (from 1 to 6 months)	effect	not classified	Not allowed
		lack of effect		Working staff, adult and child population
	Sensitizing effect (skin / respiratory)	lack of effect	4 (not classified)	Working staff, adult and child population
		(lack of effect)		
	Conformity assessment the content of the DV medium (C) on the skin with a hygienic standards	C / PDU (ODE) on the skin ≤1	3-4	Working staff and population in everyday life
	Clinical trials pilot party repellent with using a new DV	Absence of reactions. Absence of changes compared with control and background	Not classified	Production Recommendation and registration of funds
1.8.1.2. Aerosol	Acute toxicity with	> 2500	4	1) Working staff

cylinders, BAU	application to skin (for BAU) (DL 50 , mg / kg).	(> 5000)	classified) (not	adult and 1) Working staff 2) children's population

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Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Classification assessment of	Allowed application of
	Acute toxicity with in the stomach (for BAU), (DL 50 , mg / kg).	> 150 (> 300 - ≤ 5000)	3-4 (4-5)	Working staff and adult population in regulation of conditions applications
		> 5000 (> 5000)	4 (not classified)	Working staff, adult and child population
	Acute inhalation danger in saturating the concentration of vapor (C_{20})	With $_{20}$ = Lim $_{ac}$	3	Working staff adult population in regulated conditions of use.
		With $_{20}$ <Lim $_{ac}$	4	Working staff adult and child population
	Acid biocidal zone effect	31 - 100	3	1) Working personnel and population in everyday life wi regulated conditions of use.
	Acid biocidal zone effect (the indicated "zones" for alcohol-containing products decreases by an order of magnitude)	> 100	4	Working staff, adult population in everyday
	Irritant effect on skin during repeated applications (0.5-1 months)	weak		Working staff in regulated mode applications
		no effect is not classified		Working staff, adult population. Children's population (for children use funds from

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Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Classification assessment of	Allowed application of
				content of DV not more than %)
	Acute irritating action on the eyes, scores	4.0-6.0	3	Working staff
		0 - 3.0	4-5	Working staff, adult and child population
	Skin resorptive and irritating effect on skin (4 to 6 months)	effect	not classified	Not allowed
		lack of effect		Working staff adult and child population

	Sensitizing effect (skin / respiratory)	weak (low)	3B (1B)	Working staff
		lack of effect (lack of effect)	4 (not classified)	Working staff, adult and child population
	Conformity assessment content solution (C) in the air hygienic standards (conducted with necessity)	C / MPC (OBUV) r.z. ≤1	2-4	Working staff and population in everyday life
		C / MAC (OBUV) as. ≤1		
		C / PDU (TAC) for skin ≤1		
	Clinical trials pilot party repellent with using a new DV	Absence of reactions. Absence of changes compared with control and background	Not classified	Production Recommendation and registration of funds
1.8.2. Repellent means for impregnation	Acute toxicity with application to the skin (DL 50 ,	> 2500 (> 2000)	4 4-5	1) Working personnel, adult and 2) child

Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Classification assessment of	Allowed application of
and processing of clothing and fabrics, for application to surface, coating (solutions, emulsions, pencils, etc.)	mg / kg)			population
	Acute toxicity with introduction into the stomach, (DL 50 , mg / kg).	> 150 (> 300 - ≤ 5000)	3-4 (4-5)	Working staff and adult population in regulation of conditions applications
		> 5000 (> 5000)	4 (not classified)	Working staff, adult and child population
	Skin and oral coefficient	<3		Working staff.
		> 3		Working staff, adult and child population
	Acute inhalation danger in saturating the concentration of vapor (C 20)	With 20 = Lim ac	3	Specialists and adults population in everyday life wi regulated conditions of use.
		With 20 > Lim ac	4	Specialists and population in everyday life
	Inhalation hazard Vapor from tissues treated means.	effect	3	Specialists with regulated terms of use
		lack of effect	4	Working staff, adult population
	Acute irritating / corroding effect on skin, scores (erythema, edema)	2.1 - 4.0 (≥ 1.5 - <2.3)	3 (3)	Specialists with regulated conditions of use.
		0 - 2.0 (<1.5)	4 (not classified)	Working staff, adult and child population

Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Classification assessment of	Allowed application of
	Acute irritating action on the eyes, scores	4.0-6.0	3	Specialists regulated terms of use
		0-2.0	4-5	Working staff, adult population
	Skin resorptive and irritating effect on skin (4 to 6 months)	effect lack of effect	not classified	Not allowed Specialists and population in everyday life
	Sensitizing effect (skin / respiratory)	weak (low) lack of effect (lack of effect)	3B 1B 4 (not classified)	Working staff Working staff, adult population
	Toxicity analysis for bull spermatozoa (stretch from the fabric)	toxicity index 70-120%		Staff, patients and population in everyday life
	Clinical trials pilot party repellent with using a new DV	Absence of reactions. Absence of reliably significant changes compared with control and background	Not classified	Production Recommendation and registration of funds

1) Working personnel (loggers, geologists, ameliorators, etc.).
2) Repellent means for young children (from one year of life and older) must be only 4 classes with limited criteria (when applied to the skin, inhaled vapors in saturating concentrations, EHF - more than 3), without long-term effects, with absence of irritating, resorptive, sensitizing and immunomodulating effects, as well as the presence of a remote control for DV on skin and with a safety factor (more than 10).

1.9. Pediculicidal means

Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Classification assessment of	Allowed Application
1.9.1. Pediculicidal means for fighting head and pubic lice				
1.9.1.1. Means in the form of concentrate	Acute toxicity with introduction into the stomach (DL_{50}, mg / kg)	> 151 (> 50)	3-4 (3-5)	Specialists and population in everyday l
	Acute toxicity with application to the skin (DL_{50}, mg / kg).	<500 (≤ 1000)	1-2 (1-3)	Prohibited Specialists and population in everyday l
		> 2500 (> 1000)	4 (4-5)	
	Acute inhalation danger in saturating the concentration of vapor (C_{20})	C_{20} - clinic With $_{20}$ > Lim_{ac}		Specialists with use of PPE (respirator, protective glasses, rubber gloves) For specialists and population in everyday l
	Acute irritating / corroding effect on skin, scores (erythema, edema)	> 4.0 (≥ 2.3)	1-2 (1-2)	Prohibited To specialists population in everyday l
		2.1 - 4.0 (≥ 1.5 - <2.3) (<1.5)	3-4 (3) (not classified)	

1.9.1.2. Ready for application of the pediculicide drugs (lotions, gels, shampoos, soap, dust or work solutions)	Acute toxicity with introduction into the stomach (DL 50 , mg / kg)	> 150 (> 300)	3-4 (4-5)	Specialists and population in everyday l
	Acute toxicity with application to the skin (DL 50 , mg / kg).	<2500 (≤ 1000)	1-3 (1-3)	Prohibited

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Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Normative indicators: Classification assessment of	Allowed Application
		> 2500 (> 1000)	4 (4-5)	Specialists and population in everyday l
	Acute irritating / corroding effect on skin, scores (erythema, edema)	2.1-6.0 (≥ 1.5)	2-3 (2-3)	Prohibited
		0 - 2.0 (<1.5)	4 (not classified)	Specialists and population in everyday l
	Acute irritating action on the eyes, scores	4-6	3	Specialists and population in everyday l regulated terms of use
		0-3	4-5	No limits
	Skin Resorptive action (21/28 days)	effect	not classified	Prohibited
		lack of effect		Specialists and population in everyday l
	Sensitizing effect (skin / respiratory)	lack of effect (lack of effect)	4 (not classified)	Specialists and population in everyday l
1.9.1.3. Aerosol cylinders, BAU	Acute toxicity with application to the skin (DL 50 , mg / kg)	<2500 (≤ 2000)	1-3 (1-4)	Prohibited
		> 2500 (> 2000)	4 (5)	Specialists and population in everyday l
	Acute irritating / corroding effect on skin, scores (erythema, edema)	2.1-6.0 (≥ 1.5)	2-3 (2-3)	Prohibited
		0 - 2.0 (<1.5)	4 (not classified)	Specialists and population in everyday l
	Acute irritating action on the eyes, scores	4-6	3	Specialists and population in everyday l regulated terms of use

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Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Normative indicators: Classification assessment of	Allowed Application
		0-3	4-5	No limits
	Skin Resorptive action (21/28 days)	effect	not classified	Prohibited
		lack of effect		Specialists and population in everyday l
	Sensitizing effect (skin / respiratory)	moderate / weak (moderate / low)	3A / 3B (1B)	Prohibited
		lack of effect (lack of	4 (not classified)	No limits

		effect)		
1.9.2. Means for combating lice				
1.9.2.1. Ready for application of the pediculicide drugs (solutions, emulsions, dusts)	Acute toxicity with introduction into the stomach (DL 50 , mg / kg)	> 150 (> 300)	3-4 (4-5)	Specialists and The population in every
	Acute toxicity with application to the skin (DL 50 , mg / kg)	<2500 (≤ 2000)	1-3 (1-4)	Prohibited
		> 2500 (> 2000)	4 (5)	Specialists and population in everyday l
	Acute irritating / corroding effect on skin, scores (erythema, edema)	2.1-6.0 (≥ 1.5)	2-3 (2-3)	Prohibited
		0 - 2.0 (<1.5)	4 (not classified)	Specialists and population in everyday l
	Skin Resorptive action (21/28 days)	effect	not classified	Prohibited
		lack of effect		To specialists population in everyday l
	Sensitizing effect (skin / respiratory)	moderate / weak (moderate / low)	3A / 3B (1B)	Specialists with regulated terms of use
		lack of effect	4	Specialists and

Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Classification assessment of	Allowed Application
		(lack of effect)	(not classified) to the population in everyday life	
	Inhalation toxicity with repeated exposure (14 days) in the application modes:		not classified	
	in the norm of consumption	effect		Prohibited
	in 3 rates of consumption	effect		Specialists with use of PPE (respirators, protective glasses, rubber gloves)
	in 10 rates of consumption	effect		Specialists and population in everyday l regulated terms of use
		lack of effect		Specialists and population in everyday l
	Definition organoleptic indicators of materials, impregnated with pediculicide drugs, in accordance with the requirements MUK 4.1 / 4.31485-03 "Hygienic evaluation clothes for children, teenagers and adults »	non-conformity		Application by epidemic indications
		conformity	not classified	Specialists and population in everyday l

1.10. Disinfectants

Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Normative indicators: Classification assessment of	Allowed application of
1.10.1. Dedication funds in various forms (pastes, gels, oil solutions, dough)	Acute toxicity with introduction into the stomach (DL $_{50}$, mg / kg)	> 15 (> 5 - ≤ 2000)	2-4 (2-4)	Specialists with use of PPE (latex gloves, protective glasses, overalls, etc.)
		> 5000 (> 2000)	4 (5)	No limits
	Acute toxicity with applying to the skin, DL $_{50}$, mg / kg	> 500 (> 200 - ≤ 2000)	3-4 (3-4)	Specialists with use of PPE (latex gloves, protective glasses, overalls, etc.)
		> 2500 (> 2000 - ≤ 5000)	4 (5)	No limits
	Cumulative effect (by classification of Bear LI):			
	- anticoagulants	≤ 3	1-2	No limits
	- poisons of acute type of action	> 3	3-4	No limits
	Acute inhalation danger in saturating concentrations (C $_{20}$)	With $_{20}$ ≥ Lim $_{ac}$	2-3	Specialists with use of PPE (respirators, rubber gloves, goggles, overalls, etc.)
	Skin Resorptive action (21/28 days)	With $_{20}$ <Lim $_{ac}$ effect	4 not classified	No limits Specialists with use of PPE (latex gloves,

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Appointment facilities	Subjects indicators	Normative indicators: Value indicator	Normative indicators: Classification assessment of	Allowed application of
				overalls, etc.) Specialists, population in everyday l
	Irritant effect on skin during repeated applications (0.5-1 months)	lack of effect moderate / weak	not classified	Specialists with use of PPE (latex gloves, overalls, etc.) Specialists, population in everyday l
1.10.2. Deratizing means in the form of dusts, powders, powders	Acute toxicity with introduction into the stomach, (DL $_{50}$, mg / kg)	lack of effect > 15 (> 5 - ≤ 2000)	2-4 (2-4)	Specialists with use of PPE (latex gloves, protective glasses, overalls, etc.)
		> 5000 (> 2000)	4 (5)	To specialists, to the po at home
	Acute toxicity with application to the skin (DL $_{50}$, mg / kg)	> 500 (> 200 - ≤ 2000)	3-4 (3-4)	Specialists with use of PPE (latex gloves, protective glasses, overalls, etc.)

		> 5000 (> 2000)	4 (5)	To specialists, to the po at home
	Cumulative effect (by classification of Bear LI):			
	- anticoagulants	not more than 3	1-2	Specialists and
	- poisons of acute type of action	more than 3	3-4	population in everyday l Specialists and population in everyday l

		Normative indicators		
Appointment facilities	Subjects indicators	Value indicator	Classification assessment of	Allowed application of
	Conformity assessment content	C / MPC (OBUV) r.z. ≤1	2-4	To specialists
	solution (C) in the air hygienic standards (conducted with necessity)	C / MAC (OBUV) as. ≤1	3-4	The population in every
	Acute irritating action on the eyes, scores	<4.0	3-4	Specialists and population in everyday l regulated terms of use
		0	5	Specialists and population in everyday l
1.10.3. Deratizing baits (cereals, granular blocks wax, paraffined, etc.)	Acute toxicity with introduction into the stomach (DL 50 , mg / kg)	> 150 (> 300 - ≤ 5000)	3-4 (4-5)	Specialists with use of PPE (latex gloves, protective glasses, overalls, etc.)
		> 5000 (> 5000)	4 (not classified)	Specialists and population in everyday l
	Cumulative effect (by classification of Bear LI):			
	- anticoagulants	≤ 3	1-2	Specialists and population in everyday l
	- poisons of acute type of action	> 3	3-4	Specialists and population in everyday l

Note to the tables 1.1-1.10.

* Classification assessment - hazard classes are indicated in accordance with P 4.2.2643-10 and in accordance with GOST 12.1.007-76 (valid until acceptance of classific harmful substances in accordance with the GHS). Classification by GHS is shown in parentheses.

** Specialists: personnel trained in the field of disinfection.

2. Physicochemical indicators of disinfection means

2.1. Disinfecting, sterilizing agents and means for pre-sterilization cleaning

No. p / p	Assignment of funds	Investigated indicators	Normative indicators
1	Substances for the production of disinfectants, sterilizing agents and means for pre-sterilization treatment	Appearance (aggregate state, color), odor, physicochemical properties (hydrogen index (pH), etc.), mass	Established in GOST. Specification and sp manufacturers

		active ingredient fraction	
2	Disinfectants in different forms application (liquid, powder, tablet, paint, varnishes, pastes, aerosol cans, gels, etc.)	Appearance (aggregate state, color), odor, physicochemical properties (hydrogen index (pH), etc.), mass active ingredient fraction	Established in GOST, Specification and s manufacturers
3	Sterilizing agents in different forms of application (liquids, gases, vapors, powders, etc.)	Appearance (aggregate state, color), odor, physicochemical properties (hydrogen index (pH), etc.), mass active ingredient fraction	Established in GOST, Specification and s manufacturers
4	Means for pre-sterilization cleaning in different forms of application (liquids, powders, granules, etc.)	Appearance (aggregate state, color), odor, physicochemical properties (hydrogen index (pH), etc.), mass active ingredient fraction	Established in GOST, Specification and s foreign firms on products

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2.2. Disinsection means

No. p / p	Assignment of funds	Investigated indicators	Normative indicators
1	Substances for production insecticidal, pediculicidal, repellent and of acar-insecticidal agents	Appearance (aggregate state, color), odor, physicochemical properties (hydrogen index (pH), etc.), mass active ingredient fraction	Established in GOST, Specification and s manufacturers
2	Insecticides in aerosol cans	Appearance (aggregate state, color), odor, physicochemical properties (hydrogen index (pH), etc.), mass active ingredient fraction	Established in GOST, Specification and s manufacturers
3	Insecticides in other forms of application (concentrates of emulsions, wettable powders, gels, food baits, dust, bait, checkers)	Appearance (aggregate state, color), odor, physicochemical properties (hydrogen index (pH), etc.), mass active ingredient fraction	Established in GOST, Specification and s manufacturers
4	Pediculicidal agents in different forms of application (lotions, shampoos, emulsion concentrates, soaps (solid, liquid), creams	Appearance (aggregate state, color), odor, physicochemical properties (hydrogen index (pH), etc.), mass active ingredient fraction	Established in GOST, Specification and s manufacturers
5	Repellents in aerosol containers	Appearance (aggregate state, color), odor, physicochemical properties (hydrogen index (pH), etc.), mass active ingredient fraction	Established in GOST, Specification and s manufacturers
6th	Electrofumigating repellent means (plates, spirals, liquids, candles)	Appearance (aggregate state, color), odor, physicochemical properties (hydrogen index (pH), etc.), mass active ingredient fraction	Established in GOST, Specification and s manufacturers
7th	Repellents in other forms of application (lotions, emulsions, gels, milk, creams)	Appearance (aggregate state, color), odor, physicochemical properties (hydrogen index (pH), etc.), mass active ingredient fraction	Established in GOST, Specification and s manufacturers

No. p / p	Assignment of funds	Investigated indicators	Normative indicators
8	Akaro-insecticidal agents (aerosol cans, concentrates of emulsions, pencils)	Appearance (aggregate state, color), odor, physicochemical properties (hydrogen index (pH), etc.), mass active ingredient fraction	Established in GOST. Specification and sp manufacturers

2.3. Disinfectants

No. p / p	Assignment of funds	Investigated indicators	Normative indicators
1	Substances for the production of deratization products	Appearance (aggregate state, color), odor, physicochemical properties (hydrogen index (pH), etc.), mass active ingredient fraction	Established in GOST Specification and s manufacturers
2	Deratization means in different forms of application (grain baits, granules, gels, etc.	Appearance (aggregate state, color), odor, physicochemical properties (hydrogen index (pH), etc.), mass active ingredient fraction	Established in GOST Specification and s manufacturers

List of abbreviations used

Lim $_{ac}$ - threshold of acute action;

Lim $_{subac}$ - threshold of subacute action;

C $_{20}$ - saturation concentration of vapor at 20 ^{0} C;

BAU - non-propellant aerosol packaging;

DV - an active substance;

DOC - allowable residual quantities;

DS - disinfection means;

DDD is the permissible daily dose;

Medical devices;

K $_{к / о}$ - dermal-oral coefficient;

To $_{cum}$ - cumulation factor (Bear by classification LI);

LD $_{50}$ (DL $_{50}$) is an average lethal dose;

LK $_{50}$ (CL $_{50}$) - medium-lethal concentration;

MЭOK - minimum effective disinfecting concentration;

TSEL $_{AV}$ - an approximate safe level of exposure in the atmospheric air of populated areas;

OBUV $_{r.z.}$ - approximate safe level of exposure in the air of the working area;

ODE $_{in.}$ - approximate permissible level in water of water objects of domestic and drinking and cultural and domestic water use;

ODU $_{z.kp.}$ - approximate allowable level of skin contamination;

MPC $_{AV}$ - the maximum permissible concentration in the atmospheric air of populated areas;

MAC of the $_{bass.}$ - the maximum permissible concentration in water of swimming pools and water parks;

MPC $_{in.}$ - maximum permissible concentration in water of water objects of domestic and drinking and cultural and domestic water use;

PDU $_{z.kp.}$ - maximum permissible level of skin contamination;

MPC $_{r.z.}$ - the maximum permissible concentration in the air of the working area;

GHS - Globally Harmonized Globally Harmonized System of Classification and Labeling of Chemicals

System of Classification and Labeling of Chemicals - GHS);

SC $_{50}$ - concentration of active substance, causing 50% of death of individuals;

CTP is a cytotoxic action.

Appendix 20.2 to Section 20
Single sanitary-
epidemiological and hygienic
requirements for products (goods),
subject to sanitary-
epidemiological surveillance
(control)

Unified methods of research effectiveness
disinfectants

Introduction

The document "Unified methods of effectiveness research Disinfectants "was developed in accordance with the Decision Collegium of the Eurasian Economic Commission No. 35 of April 19 2012 "On approval of the" Regulation on the unification of methods tests for the purpose of assessing compliance with a single sanitary-epidemiological and hygienic requirements for goods, subject to sanitary and epidemiological supervision (control) "with the purpose of forming and approving the Unified list of methods (methodologies) for the purposes of assessing the conformity of products to the Single sanitary and hygienic requirements.

Unification of research methods and evaluation criteria The effectiveness of disinfection means is necessary to ensure Uniformity of requirements and comparability of test results disinfectants of various composition during the disinfection expert examination for the purpose of state registration in the customs territory of the Union, held in different countries of the Union, as well as to ensure the standardization of conditions

use of equivalent disinfectants (similar
composition).

The basis of the draft document on Unified Methods of Research
The effectiveness of disinfection
principles:

- use only officially approved methods of one
from the Parties;

- Recognition of methods / methodologies officially approved
parties;

- mutual recognition of metrological attestation (confirmation
fitness) methods;

- mutual exchange of methods / test procedures;

- the possibility of updating the Unified list of methods / techniques and
making changes and additions to it.

This document is developed on the basis of Guideline R 4.2. 2643-10
"Methods of laboratory research and testing of disinfection
means to assess their effectiveness and safety "(M., 2010), and
also at accordance from official national
(state) methods of conducting research in
Republic of Belarus, partially harmonized with the European
Union, as well as taking into account the suggestions and comments of the Republic
Kazakhstan.

Unified methods for investigating the effectiveness of disinfection
means include:

microbiological research methods and evaluation criteria
effectiveness of disinfectant and sterilizing agents (including
number of methods used in the Republic of Belarus);

entomological research methods and evaluation criteria
effectiveness of disinfestations;

rodentological research methods and evaluation criteria

effectiveness of deratization means.

1. Microbiological research methods and evaluation criteria effectiveness of disinfectants and sterilizing agents

Modern disinfectants (hereinafter - DS)

are individual chemical compounds or

Compositions comprising one or more

active substances (hereinafter - DV). In addition, they may

include auxiliary components: stabilizers, inhibitors

corrosion, detergents, dyes, fragrances, etc.

As active substances in the DS, use is made of chloroactive,

Oxygen compounds, aldehydes, quaternary ammonium

compounds, amines, guanidines, alcohols, etc.

DS are produced in the form of liquid concentrates ready for

application of liquids, powders, tablets, gels, sprays, napkins,

biocidal paints and varnishes, etc.

DS should meet the following requirements:

– have sufficient antimicrobial activity in relation to

pathogenic and conditionally pathogenic species of microorganisms: bacteria,

fungi, viruses, as well as spore forms of microorganisms;

– relatively low toxicity for humans;

– το be environmentally safe;

– το be stable during storage;

– do not have a sharp unpleasant odor;

– dissolve well in water;

– do not damage processed objects;

– το have the optimal cost-quality ratio.

To some narrowly-targeted funds in addition

present individual requirements, outlined at

relevant chapters.

DS can be a universal destination, which

It is recommended to use for disinfection of many objects
different infections, or narrowly targeted, designed for
disinfection of one particular facility, for example, for disinfection
dental impressions, or air, or surfaces, etc.

The intended scope of the DS determines the volume
microbiological research needed to develop
modes of disinfection of the corresponding objects.

Microbiological studies begin only after
of obtaining the results chemical-analytical research,
confirming compliance of the funds with the requirements of the regulatory-
technical documentation (NTD): specifications - on
domestic means, specifications - on foreign.

Stages research disinfecting substances and
disinfectants. Microbiological studies
include the study of DV activity and the study of the effectiveness of DS.

When investigating substances intended for production
DS, determine the spectrum of antimicrobial activity in experiments in
vitro.

The study of the properties of DS is carried out in 3 stages:

1) in vitro determination of the spectrum of antimicrobial activity of DS and
influence on it of various factors: pH, organic substances,
temperature.

2) efficiency disinfection artificially
contaminated with test microorganisms in laboratory
conditions for the development of regimes for the use of DS depending on the
concentration of DW, exposure time, nature of the object, method
processing and other factors.

3) DC test in practical conditions for confirmation
efficiency of the developed regimes in real conditions
application in cases: the study of a means containing a new DV;
a new method of application, a significant reduction in the concentration and
time of exposure compared to previously authorized regimes and
etc.

1.1. Methods for studying and evaluating bactericidal activity
disinfectants and their substances

1.1.1. Test microorganisms for the study of bactericidal activity
DS and their substances. Requirements for test microorganisms.

When studying the bactericidal activity of disinfectants
substances and DS as test microorganisms use:

for the evaluation of bactericidal activity against
Gram-negative bacteria Escherichia coli (strain 1257) *,

Escherichia coli K12 HCTC10538,

Escherichia coli ATCC 11229,

Escherichia coli (25912), Escherichia coli (35218),

Pseudomonas aeruginosa (strain ATCC 27853),

Pseudomonas aeruginosa ATCC 15442 (DSM 939),

Proteus mirabilis ATCC 14153 (DSM 788);

for the evaluation of bactericidal activity against
Gram-positive bacteria Staphylococcus aureus (strain 906) *,

Staphylococcus aureus ATTS 6538 (DSM 799),

Staphylococcus aureus (43300),

Staphylococcus aureus (29213),

Enterococcus faecitum ATCC 6057 (DSM 2146),

Enterococcus hirae ATCC 10541 (DSM 3320).

**) the strain is used until 01.07.2015 until the results are obtained
experimental evaluation of comparative resistance to other
strains of the microorganism of the ATCC collection.*

At certification tests and expert estimation DS
A set of test microorganisms may be limited to the most
sustainable representatives of each group, which the
is established in comparative tests.

The list of test microorganisms, if it is wider than the standard one,
specified in the relevant sections on the effectiveness of DS
at disinfection of separate objects.

Conditions for cultivation of test microorganisms.

Test microorganisms are cultured on the following nutrient environments:

E. coli, P. aeruginosa and S. aureus, etc. - on casein broth, meat-peptone broth, Endo agar, casein agar, meat-peptone agar, etc. at a temperature of plus 37 ± 1 ° C for 18-24 hours.

Museum cultures of the above microorganisms store at a temperature of plus 3 ± 1 ° C in ampoules (after freeze-drying), on dense nutrient media (seeding with a prick) under a layer of sterile vaseline oil (the thickness of the layer is 1.5-3 mm), in cryovials in freezing chamber at temperatures from minus 18 ° C to minus 70 ° C, and working cultures - on sloping agar or in broth.

Test microorganisms should have typical biochemical, morphological, tinctorial, cultural and enzymatic properties inherent in this species and possess a standard resistant to standard disinfectants: solutions chloramine, peroxides hydrogen, Catamine AB - alkyldimethylbenzylammonium chloride (ADBAH), glutaraldehyde (Table 1.1).

Table 1.1.

Stability of test microorganisms for disinfectants agents

Disinfectant substance	Concentration solution by DV,%	Time of death of test microorganisms, min, not less than	
		E. coli, pcs. 1257	S.aureus, pcs. 906
Chloramine	0.020 *	5	-
	0,200 *	-	15
ADBAH	0.025	20	10
Glutar aldehyde	0.030	10	-
	0.060	-	10
Hydrogen peroxide	2,000	10	-
	3,000	-	25

Note - the sign (*) indicates that the concentration of the solution is indicated by the preparation.

Sustainability test microorganisms to reference The disinfectant is determined by the method of cambric test-objects (clause 1.1.2.2.)

Test of stability of test microorganisms is carried out at least 1 times a month. With a decrease in the resistance of cultures, they are made transplant to enriched nutrient media prior to reconstitution sustainability.

1.1.1.1. Methods of preparation of a suspension of test microorganisms. Determination of the biological concentration of test microorganisms in bacterial suspension

Cultures of test microorganisms are subjected to control of their quality. In particular, immediately before using the test-cultures for research purposes, it is necessary to make sure that Test strains grown on a nutrient medium are not contaminated extraneous microflora. To assess the growth of cultures of test strains visually scan each tube and take into account the nature and massiveness of growth, change in the color of the nutrient medium. Conducted microscopy of the smear of grown cultures stained according to Gram.

The working suspension of the test cultures is prepared from the culture of this test-strain, grown on a dense nutrient medium (MPA or casein agar) at a temperature of 37 ± 1 ° C for 18-24 hours. For Preparing the bacterial suspension, the culture is washed off with agar sterile drinking water. The resulting suspension of microbes is filtered through a cotton-gauze filter and diluted with sterile drinking water up to concentration, corresponding to the turbidity of the optical standard turbidity (the standard of turbidity produced by FGBU "NTSESMP" Ministry of HealthRussia, standard turbidity by McFarland) $2 \cdot 10^9$ microbial bodies in 1 ml. In the absence of a ready standard turbidity control of the number of microbial bodies is carried out using densitometer.

Due to the fact that the suspension can contain, along with live dead microorganisms, it is necessary to determine the bacteriological concentration actual quantities living cells at prepared suspension to, if necessary, add

corrective measures and to ensure the required levels of contamination of cambric test objects by viable microorganisms.

Determination of the biological concentration of test microorganisms are performed by the method of consecutive tenfold dilutions suspension of the test microorganism in sterile drinking water with subsequent suspension of the suspension in a petri dish with a dense nutrient medium (casein agar, endo agar, MPA). After a certain incubation time at the appropriate temperature counting the grown colony-forming units (CFU) and determine the number of viable bacteria in one ml suspension.

It is known from practice that a suspension containing such the number of living microbes, when contaminated with batistovyh test-objects provides the required (about $1 \cdot 10^5$ - $1 \cdot 10^6$ CFU / cm^2) levels their contamination with living cells of the test microbe.

1.1.1.2. Preparation of working solutions of DS and their substances

Work solutions of DS and their substances are prepared directly before carrying out the research (except when studying stability of working solutions during storage).

When studying the means produced in the form of granules, powders, tablets, etc., working solutions are used only after a full Disinfectant disinfectant (if not indicated on the the possibility of precipitation).

To assess the antimicrobial activity of substances and DS in laboratory conditions and studies of the effectiveness of DS, destined for disinfection of various objects solutions prepared on drinking water. For this, the required amount of dry DS

or liquid concentrate is introduced into a test tube (flask) and added the calculated amount of water, mix thoroughly and cover stopper. Mark the time of complete dissolution and appearance of the prepared working solution.

The temperature of DS solutions should be in the range of plus 20 ± 2 ° C (if the experimental conditions do not recommend a different temperature) regardless of the ambient temperature. For supporting a water bath is used.

When testing DS in practical conditions, working solutions prepared on drinking water at room temperature (plus 20 ± 2 ° C) or, if necessary, moderately elevated temperature (plus 45-50 ° C). A sample of the agent or a measured amount of liquid concentrate is added to a suitable container, water is added, stir and cover with a lid. Work with the solution begins after complete dissolution of the DV. Work solutions of DS in the form Tablets are prepared by adding to a metered volume of water a certain number of tablets.

Working solutions of substances and DS are prepared in compliance with the measures precautions. If DW refers to volatile substances and is dangerous for inhalation, solutions prepared in a fume hood or in a separate room, equipped with supply and exhaust ventilation in a respirator RU-60M or RPG-67 or others designed to work with chemical compounds, the skin of the hands is protected with rubber gloves, eyes - protective goggles.

The investigated DS should be stored in accordance with the requirements technical specifications or specifications; in the absence of such - in accordance with JV 3.5. 1378-03.

1.1.2. Methods of research and evaluation of bactericidal activity of DS and their substances in vitro

The aim of the research is to determine the level and spectrum antimicrobial activity of DS and their substances.

Substances intended for the production of DS must

meet the following requirements:

dissolve well in water or other solvents;

have bactericidal activity, i.e. kill bacteria, not to delay their growth;

have satisfactory organoleptic (in color, smell) and physico-chemical (by solubility, biodegradability and storage stability, etc.).

The antimicrobial activity of DS and their substances is studied suspension method or the method of cambric test objects.

1.1.2.1 Qualitative suspension method

For the preparation of solutions of DS in various concentrations of DV diluted or dissolved in drinking water, then 4.5 ml is dispensed into sterile test tubes into which 0.5 ml of a suspension of test-microorganism or broth culture containing 1×10^9 μ / ml, and mix thoroughly. At regular intervals (5 min.) For 0.5 ml of the "test-microorganism + DV" suspension is added to 4.5 ml The appropriate neutralizer is again thoroughly mixed and leave for 5 minutes. Then, 0.5 ml is added to a test tube with 4.5 ml sterile drinking water, after which 0.1 ml of this sample is added to the test tubes with 5 ml of liquid and on the surface of a solid nutrient medium. In control experiments, instead of DS solutions, a sterile

drinking water, and crops are made on Wednesday without neutralization or with neutralization.

The incubation temperature of the crops in the thermostat is 37 ± 1 ° C, the time accounting for the results of the experiment - 24-48 hours. To confirm the withdrawal biocidal action of DV from test tubes, in which there was no growth test culture, daily done by reseeding 0.5 ml in 4.5 ml of a new nutrient medium.

The results of the experiment are assessed by the presence or absence of growth microorganisms in liquid and solid nutrient medium. Comparison spend with the control of experience, which is the sowing of test-

microorganisms into a nutrient medium without the addition of DS or substance.

Effective is the concentration of the agent, in which three times repeated experiment at a certain time of action gives negative result (lack of growth of microorganisms) presence of a typical growth of the test culture in the control.

1.1.2.2. The method of the batist test objects

Preparation of batistovye test objects.

Before preparing batistovyh test objects a piece of batiste immersed for 24 hours in cold water to remove the aplature, starch. Then it is carefully washed with soap, boiled, dried and ironed. Using a needle in a cooked piece of cloth pull the threads in the longitudinal direction at a distance of 11 mm from each other, and in the transverse - at a distance of 6 mm. Over these lines batiste cut with scissors on the test objects and 50 pieces put in Petri dishes, the latter wrapped in paper and are sterilized by a steam method at plus 132 ° C (2.0 kGs / cm^2) for 20 minutes.

Contamination of cambric test objects.

Preparation of a suspension of test microorganisms (E. coli, S. aureus, P. aeruginosa, etc.) are carried out in accordance with 1.1.1.1. For contamination of sterile cambric test objects in a petri dish pour the suspension of the test microorganism at a rate of 0.5 ml per 1 test-object, evenly wetting all test objects. Petri dish cover and leave for 20 minutes. Then, in aseptic conditions, batistovye test objects, impregnated with a suspension of test-microorganisms, transfer on surface sterile filter paper (2-4 layers at the bottom of the Petri dish), cover them on top with sterile filter paper and cover the Petri dish cover. In 10 minutes. after removing excess fluid test-objects are transferred to the surface of a dry sterile filter paper in a Petri dish and cover it with a sterile sheet filter paper, dried in a thermostat at a temperature of

plus 37 ° C for 20 min. with a slightly opened lid.

Contaminated batiste test objects are stored in plates Petri in the refrigerator at a temperature of plus 4 ° C.

The shelf-life of test items contaminated with E. coli, P. aeruginosa and others - 1 day, S. aureus - 4 days.

Statement of experience.

When setting the experiments in a sterile tube with a pipette pour the required volume of the prepared disinfectant solution (at the rate of 0.5 ml per each test object) and without touching the edges flasks, lowered into it using sterile tweezers, all test-objects used in the experiment (2 for each exposure). Light swinging of the flask achieves wetting of test objects test solution. The flask is placed in a water bath with

temperature of 18-20 ° C and maintain this temperature in throughout the entire experiment. At a constant indoor temperature plus 18-20 ° C experiments can be carried out without using water bath.

The time of action of the reference solution begins with the moment of wetting all test objects with a solution. Through certain time intervals (5 minutes) with sterile tweezers or platinum loop, 2 test-subjects are removed from the solution and immersed in test tubes with 5 ml sterile solution of the corresponding neutralizer. After 5 minutes. test objects are transferred to a test tube with sterile drinking water, and after 5 min. each of the two test-objects are separately transferred to tubes with liquid nutrient The medium necessary for the cultivation of the test-microorganism.

Control is two test objects immersed for the whole period experiment in the neutralizer solution, and 2 test objects immersed for the same period in drinking water, which at the end of the experiment is transferred to a culture medium. The crops are incubated in a thermostat at temperature 37 ± 1 ° C. The results are evaluated qualitatively by absence / presence of growth of the test microorganism in the nutritious broth.

The criterion for the activity of DS and substances is 100% death
test-microorganisms (lack of growth in test samples) at
time of disinfection of S. aureus, E. coli, P. aeruginosa and others.
not more than 30 min.

1.1.2.3 Methods for eliminating bacteriostatic action
active substances

For definitions microbicidal and exceptions
bacteriostatic action of DV after exposure
it is necessary to stop the exposure of DV to the test culture.

This is achieved by using the following methods:

the use of a chemical neutralizer;

sowing in a large volume of nutrient medium;

daily transfer to new nutrient media;

use of a chemical neutralizer of the active substance.

To neutralize the active substance, which can be
transferred with the material of the test object when it is sown in a nutritious
medium, use a neutralizer - a substance that eliminates
(neutralizes) the action of a chemical agent on a microbial cell, but
Do not kill and do not delay the growth of the test microorganism. Test objects
after exposure to a chemical agent, wash in a neutralizer
or add it directly to the nutrient medium (if
It is established that the introduced concentration of the neutralizer does not kill and
does not delay the growth of test bacteria).

As neutralizers of LW from various chemical groups
apply for:

haloactive (chlorine-, bromine- and iodactive) and
oxygen (hydrogen peroxide, its complexes with salts,
peroxyacetic acid, ozone) 0.1-1.0% solutions of sodium thiosulfate;

quaternary ammonium salts (alkyldimethylbenzylammonium
chloride, didecyldimethylammonium chloride, etc.), derivatives

guanidine (polyhexamethyleneguanidine hydrochloride, chlorhexidine Bigluconate, etc.) - 0,1-1,0% solutions of sodium lauryl sulfate

(sulfonol) or sodium lauryl sulfate solutions with 10% defatted milk or a universal neutralizer (see below);

aldehydes (glutaraldehyde, glyoxal, formaldehyde, orthophthalic aldehyde) - 1.0% solution pyrosulphite (metabisulphite) sodium or a universal neutralizer (see below);

acids - alkali in an equivalent amount;

alkalis - acids in an equivalent amount;

alcohols - dilution in water to an inactive concentration;

compositional means - universal neutralizer, containing Tween 80 (3%), saponin (0.3-3%), histidine (0.1%), cysteine (0.1%). If the composition contains oxidants, the neutralizer additionally, sodium thiosulfate is added.

The procedure for controlling the completeness of neutralization of the active substance using a culture of bacteria. In order to reduce time to select the optimal neutralizer, as well as objective confirmation of the fact that DV entering into the substance or DS, completely neutralized, use a culture of bacteria, sensitive to the investigated DV.

Therefore, every case of a DS test should preliminary accompanied by experimental control effectiveness of neutralization of residual effect of DS on microbial cell, as well as bactericidal and bacteriostatic action used concentration of the neutralizer.

To monitor the effectiveness of the neutralizer and completeness neutralization DS use suspension method, which provides for the study, basic operations which and their purpose are shown in Scheme 1.1 and in Table 1.2.

Scheme 1.1.

Conducting an experiment to control the effectiveness of neutralization

The action of the DS on E. coli used by the neutralizer

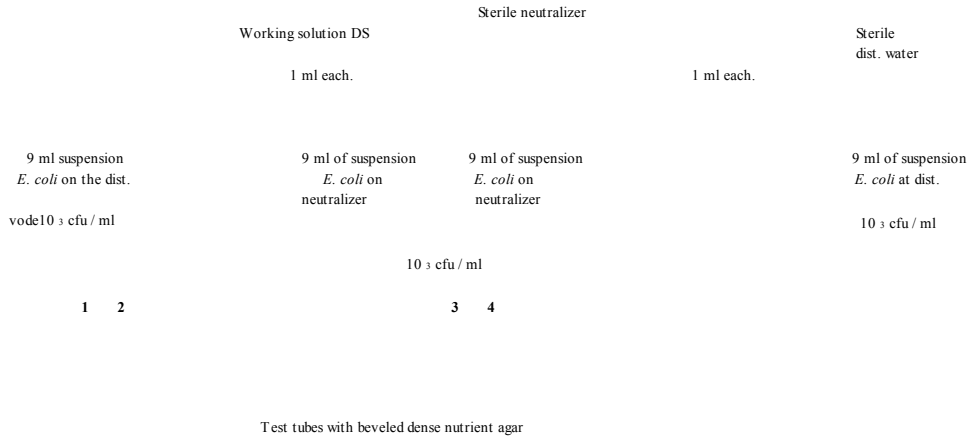

Table 1.2.

Assignment of experiment operations for evaluation effectiveness of neutralization of residual effect of DS

No. samples	Purpose of the operation research	Execution procedure research operations	Expected result
1	Control of the destructive DS actions	to 9 ml of a suspension of test-culture (10 3 cfu / ml) at dist. water + 1 ml of solution DS	Growth microorganisms must absent
2	Control of completeness neutralizing the DS	to 9 ml of a suspension of test-culture (10 3 cfu / ml) at neutralizer + 1 ml solution of DS	About the same number of colonies in the samples 0.1 ml) on a dense nutrient medium
3	Control of absence antimicrobial effect in the neutralizer	to 9 ml of a suspension of test-culture (10 3 cfu / ml) at neutralizer + 1 ml neutralizer solution	
4	Reference Control the number of bacteria (*E. coli*)	to 9 ml of a suspension of test-culture (10 3 cfu / ml) at d.v. + 1 ml dist. Water	

Note: 5 minutes after the experiment, from each of the four samples produce a mixture of 0.1 ml, at least 3 tubes with beveled a nutrient medium that is incubated in a thermostat at 37 ◦ C, after 2-3 days take into account the results of research.

Criteria for the selection of active substances (substances).

Criterion for selecting the active substance as a substance

to create a DS is the presence of bactericidal activity.

The death of test microorganisms should be 100% at

time (min.) DS in the minimum concentration in relation to
bacteria not more than 30 min.

1.1.2.4 Methods for studying factors affecting
on bactericidal activity of DS and their substances

Studies include the determination of the spectrum of antimicrobial
actions DS, and when conducting in-depth study -
in addition, the influence of various factors (pH, temperature,
organic substances) on the antimicrobial activity of solutions of DS.
This need arises in the study of DSs created on the basis of
a new, previously unexplored DV.

A study of the spectrum of antimicrobial activity and the effect on
the activity of various factors is carried out using the batist test-
objects (clause 1.1.2.2.), contaminated with test microorganisms.

A study of the dependence of the activity of DS on the presence
organic substances are carried out when added to the suspension
microorganisms 20% inactivated horse serum or
Serum of cattle with a contamination of bathist test-
objects.

To inactivate the normal serum, it is heated on
water bath at a temperature of plus 56 ° C for 30 minutes.

The bactericidal activity of DS in the presence of
organic substances are carried out with those concentrations that

were effective at disinfection of test objects,
contaminated with test microorganisms without the addition of serum.

If the activity of the agent under such experimental conditions is not
The concentration of serum is increased to 40%. When
no decrease in the activity of DS and with the addition of 40% protein
it is believed that the activity of the agent does not decrease in the presence of protein.

The effect of temperature on the activity of the DS under study is studied
at disinfection of contaminated cambric test objects
solutions of agents at different solution temperatures (from minus

30 to plus 50 ° C). To this end, a flask with a test solution of DS is placed in a water bath or refrigerating compartment and installed necessary temperature. The temperature of the solution is adjusted to of the desired level and the contaminated test-microorganisms, batistovye test objects. Further order experience such as in the study of the spectrum of antimicrobial the action of substances (1.1.2.2.). The temperature is maintained for preset exposure. If the effective concentration is the same for temperature of solutions plus 18-20 ° C and other values, that the temperature does not affect the activity of the DS and, accordingly, if the effective concentration increases or decreases, then consider that with an increase or decrease in temperature the effectiveness of DS decreases or increases.

A study of the effect of the pH of the medium on the activity of the DS are studying at disinfection contaminated test-microorganisms of cambric test objects in solutions of DS with different pH values. A number of dilutions of the test substance artificially acidifying or alkalizing solutions. For acidification use decinormal solution of hydrochloric or other acid, and

for alkalinization - decinormal alkali solution. Order of experience is the same as in assessing the spectrum of antimicrobial actions of substances (clause 1.1.2.2.).

The influence of environmental factors on the activity of DS is taken into account when the development of optimal regimes and the application of DS in practice.

1.1.3 Methods of investigation of bactericidal effectiveness of DS, intended for disinfection of objects of the external environment, contaminated with test microorganisms

The purpose of the study is the development of regimes for the use of DS with conditions of their further application in practice for disinfection Medical products (MI), items for nursing, toys, linen, surfaces, dishes, vydeleny, etc., depending on the type contamination, concentration of active substance, time impact, the rate of consumption, the nature of the object, the presence on it

organic pollution and its specificity, temperature, method and
fold processing.

1.1.3.1 Study of bactericidal effectiveness of DS,
intended for the disinfection of medical devices

When choosing a DS for research with the indicated purpose, it follows that
consider:

purpose and multiplicity of medical devices
(multiple or single use);

The presence of negative properties of DS, for example, corroding
or fixing action, etc., limiting the possibilities of its
use or require an individual methodical
approach in research;

The material (materials) from which the medical
product;

functional features of the product and its conditions
operation, which determine the specifics of the methodology
experiment and subsequent recommendations on their technology.
disinfection.

A study of the effectiveness of DS intended for
disinfection of medical products from various materials
(except endoscopes). As test products use sterile
tools and medical devices (catheters, micropipettes,
plastic spatulas, etc.) from various materials (metals,
rubber, glass, plastic) or simulating their test objects. Scroll
tools taken in the experiment should include at least three
tools with lock parts (forceps, scissors, corncang)
and not less than two, not having lock parts (tweezers, spatulas), and
also dental, including rotating, tools are not
less than two (boron, drilling root, mirror, disk grinding). AT
As test products from rubbers, glass, plastics are used
fragments of products (catheters, micropipettes, spatulas, etc.).

As test microorganisms, S. aureus is used,

P. aeruginosa. On the surface of the test-product (for medical locks products - in the castle area, and in the presence of channels and cavities - also in the channel product) using a pipette is applied to 0.1 ml of 1 billion suspension one or another type of test microorganism containing 40% inactivated horse serum. Test-products are dried until completely dry. Small test products are immersed in said suspension of test microorganisms for 15 minutes, then they are removed and dried (until completely dry). When testing DS,

with fixing properties, the amount of added the serum is reduced to 5%.

Disinfectant solutions are prepared on drinking water. After the contaminated products are completely immersed in solution of the tested DS, filling it with all channels and cavities of products, avoiding the formation of air congestion. Tools that have lock parts, immerse open, previously making them into the DS solution several working movements for better penetration solution in hard-to-reach areas of products in the lock area. Thickness layer of solution DS over products should be at least 1 cm. In parallel to control the product is immersed in water.

After a certain time (from 5 to 120 minutes), the products are removed from the disinfectant solution and gauze 5x5 cm^2, impregnated with a neutralizer, flushes are made from the surface of the product, then the napkin is placed in a test tube with 10 ml of the same neutralizer and shake with beads for 5-10 minutes. The product channel is washed neutralizer solution. Small articles are immersed in a solution neutralizer for 5 minutes, and then transferred to tubes with a liquid nutrient medium. To control the effectiveness of disinfection flushing liquid from the surface of the article and from the channel are sown on appropriate nutrient media. Crops are maintained in Thermostat with temperature and time optimal for growth used test-microorganism.

The multiplicity of the statement of the experiment must be sufficient for obtaining statistically reliable results.

The regime (concentration-time-temperature) is considered to be effective; ensuring the death of the test microorganism on all products.

In the presence of positive samples, the experiment is repeated, increasing concentration or time of exposure.

The criterion of decontamination efficiency is 100% microorganism.

Time disinfection medical products, contaminated S. aureus, P. aeruginosa - not more than 60 min.

Study of bactericidal effectiveness of DS, intended for disinfection of endoscopes, including high level disinfection.

As test objects, sterile fragments are used endoscope or endoscope (flexible - gastroscope, hard - cystoscope), and in as a test microorganism - S. aureus, P. aeruginosa.

On the outer surface of the test object, 0.1 ml of 1 mlrd. a suspension of a test microorganism containing 5% serum; across the endoscope channel is pipeted with at least 5 ml of such the same suspension. After that, the endoscope is dried for 20 minutes. The contaminated product is then immersed in the DS solution, filling cavities and channels of the endoscope. At regular intervals in during 5-60 minutes. the product is removed from the solution and flushed with outer surface with a gauze cloth moistened in a solution neutralizer. The product channel is washed with a neutralizer. Flushing the liquid is inoculated into appropriate nutrient media.

The criterion for the effectiveness of endoscope disinfection is 100% death of the test microorganism. Time of disinfection of endoscopes, contaminated S. aureus, P. aeruginosa - not more than 60 min.

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1.1.3.2 Study of bactericidal effectiveness of DS, intended for disinfection of dental impressions.

DS, intended for disinfection of dental

It should have a wide range of antimicrobial activity, not cause changes in the properties and sizes of the impressions, have a short time of disinfection (no more than 30 minutes).

In the development of regimes for disinfection of dental

Prints as test microorganisms use S. aureus, P. aeruginosa, as test objects - impressions from alginate, silicone or other materials. For the production of impressions The impression mass obtained in accordance with the recommendations are placed in a plastic or metal spoon and make an impression from plastic dentures with a modeled gum. On the prints, apply 0.1 ml of 1 mrdd. suspension test-microorganism (with the addition of 40% inactivated serum), dry them for 2-3 minutes, then completely immerse in solution of DS. With the fixed fixing action of the DS, the impressions Before immersion in a disinfectant solution, wash with a flowing drinking water. In parallel for monitoring, contaminated Impressions are immersed in water. After a certain time (5-30 minutes) The impressions are removed from the solution and a gauze cloth soaked in neutralizer, make rinses. The napkins are placed in sterile tubes with beads containing 10 ml of neutralizer, and shaken in for 10 minutes. Then, the flushing liquid is applied to nutrients environment to control the effectiveness of decontamination. Criterion efficiency of disinfection - 100%.

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1.1.3.3 Study of bactericidal effectiveness of DS, intended for disinfection of care items for ill, toys

As test objects, care products are used
patients (liners, rubber heaters, ships, thermometers,
plastic tips for enemas, etc.), toys (plastic,
metal, wooden, rubber, other than soft) or test-
objects that imitate them. As test cultures are used
S. aureus, E. coli and others.

Before contamination by test microorganisms, test objects
subject to mechanical cleaning - wash with water and soap and a brush. For
imitation pollution is used by 40% inactivated horse
serum or serum of cattle. For this, before
contamination of objects to a suspension of test microorganisms is added
the required amount of serum.

After drying contaminated test objects
are placed horizontally and a suspension of test-
microorganisms at the rate of 0.5 ml 2 billion microbial suspensions per
area of 100 cm^2 . The suspension is evenly distributed over
surface of test objects with a glass spatula, dried (before
complete drying) at room temperature plus 18-20 ° C and
relative air humidity of 50-60%, then treated
disinfecting solution.

The treatment of nursing and toys is carried out
methods of wiping, immersion, and for large toys - in a way
irrigation (drip).

The rate of disinfectant solution consumption during disinfection
methods of wiping or irrigation are determined depending on the

Method of processing is similar to experiments on disinfection
surfaces (Section 5.1.3.5.). Double wiping or irrigation
spend 5-15 minutes. after the first.

When treated by immersion in a disinfectant solution
items for nursing and small toys the latter should
completely and with an excess of covering all objects. When immersed
small toys must be prevented from ascending.

The time for disinfection of objects is determined in the interval from 15 to

120 min. depending on the type of test microorganism and the availability of organic pollution.

Control test objects are treated with sterile drinking water from the same calculation as the experimental ones.

Control of the effectiveness of disinfection of contaminated Test objects are performed as follows: gauze tissue (5×5 cm^2), wetted in neutralizer solution corresponding to the given DS, thoroughly wipe the test object and immersed in 10 ml of the same neutralizer in test tubes with beads. Wash time for gauze napkins 10 min. at constant shaking. The washing liquid is sown for 2-3 cups 0.2-0.5 ml per each on solid nutrient media.

The crops are placed in a thermostat at a temperature of plus 37 ± 1 ° C and take into account the results after 2 days.

The criterion of decontamination efficiency is not less than 100%.

Disinfection time (min.) Of objects contaminated with S.aureus, E.coli and others i - not more than 60 min.

1.1.3.4 Study of bactericidal effectiveness of DS, intended for disinfection of linen

Effectiveness of disinfection of DC linen is determined with using test objects, which are pieces of fabric from a calico 2×2 cm. As test cultures, S. aureus, E. coli and other

When developing the disinfection regime for contaminated suspensions of microorganisms before contamination of test objects 40% of inactivated serum (6 ml of 2 billion suspensions the test cultures are mixed with 4 ml of inactivated serum) or 40% of the fecal emulsion (6 ml of a 2-billion suspension of test culture mixed with 4 ml of fecal emulsion). For cooking fecal emulsions 8 g of faeces are ground in a mortar with 20 ml of water. The contaminated test objects are dried in a thermostat with a plus

37 ± 1 ° C for 20-25 minutes. or 1.5-2 hours at room temperature
until completely dry.

Sterile test objects are impregnated with a 2-billion suspension of test-microorganisms at the rate of 20 ml for 10 test objects and dried in a thermostat. Further, the contaminated test objects are placed in the Sterile coarse calico bags of 5x8 cm size, 2 pieces each.

When developing the disinfection regimes for clothes that are not contaminated secretions, the laundry is immersed in a container with a disinfectant solution at the rate of 4 liters of solution per 1 kg of dry laundry. When developing modes of disinfection of laundry contaminated with secretions, its immersed in a container with a disinfectant solution at a rate of 5 liters solution per 1 kg of dry laundry. Linen is immersed in a solution one after the other, making sure that things did not form air cavities, preventing

process of disinfection. Pouches with contaminated test-objects are distributed between the layers of laundry (top, middle, at the bottom). After a predetermined exposure time of the DS (for example, 15, 30, 60 min.) remove 1 bag from each level, with sterile tweezers remove the test objects, transfer them to the neutralizer solution at 5 minutes, then washed for 5 minutes. in sterile drinking water, then is placed in liquid nutrient media. In control experiments, instead of The sterilized drinking water is used for disinfecting solution.

The criterion of effectiveness is 100% death of the test culture.

Disinfection time (min.) Of laundry without visible impurities, contaminated S. aureus, E. coli, etc. - not more than 120 min.

Disinfection time (min.) Of laundry contaminated with secretions and contaminated S. aureus, E. coli, etc. - no more than 240 min.

1.1.3.5 Study of bactericidal effectiveness of DS, intended for disinfection of surfaces

Studies are carried out depending on the type of surfaces, their position (horizontal, vertical), method and multiplicity processing.

As test surfaces, surfaces of size
10x10 cm from various materials: smooth, rough,
absorbing and not absorbing (wooden, plastered,
surfaces, painted with oil, silicate, water-emulsion
or glue paint; wallpapered with wallpaper, linoleum surfaces
coatings, surfaces of painted or unpainted metal -
stainless chromium-nickel acid-resistant steel, plastic, glass,
artificial or natural leather, surface from facing
tiles - tile and metlakhskoy, faience, etc.), but not less than 5 types

surfaces. A set of test surfaces for research is determined
purpose of the facility.

As test microorganisms, S. aureus, E. coli and others are used.

When developing the disinfection of technological
equipment and surfaces in rooms in various industries
food industry as a test surface used
non-painted metal, plastic, facing tiles (tiled,
Metlakh, faience), contaminated sanitary-
indicative and specific for enterprises of this type
industry with microorganisms.

Before contamination with the test culture, the surfaces are exposed
Mechanical cleaning - wash with water and soap and a brush
(with the exception of surfaces covered with wallpaper and painted
glue paint). The latter are wiped several times with a sterile
a napkin moistened with sterile drinking water.

The dried surfaces are placed horizontally and on them
A suspension of test microorganisms is applied by pipette at a rate of 0.5 ml
2 billion microbial suspensions per area of 100 cm^2 and evenly
distribute it over the surface with a glass spatula. Surfaces
dried (until completely dry) at a temperature of plus 18-20 ° C and
relative air humidity of 50-60%, then treated
disinfecting solution.

When studying the effectiveness of decontamination linoleum, tile
Metlakh, artificial or natural skin, glass

are placed horizontally, and the tree, painted with oil,
silicate, water-based or glue paints, surfaces,
wallpapered with wallpaper, plastic, tile and faience tiles -
vertically.

To simulate the contamination of surfaces use protein or fecal contamination: 40% inactivated serum, 40% Fecal emulsion (in the development of disinfection regimes toilets).

Surface treatment is carried out by rubbing or irrigation (large-drop and aerosol).

To determine the rate of consumption for a single treatment The disinfectant solution is applied by pipette to the surface size 10x10 cm when applying the rubbing method in an amount of 1.0, 1.5 or 2.0 ml, and with large droplet irrigation, it is applied with using a dispenser of 1.5-3.0 ml. In the aerosol treatment method study the effectiveness of decontamination at a rate of flow 30-50-100 ml / m_2 (depending on the type of aerosol generator and the aerosol nozzle). Multiple wiping or irrigation spend with an interval between treatments of 5-15-30 minutes.

The disinfection time of surfaces is determined in the range from 5 to 120 minutes. The choice of exposure depends on the purpose and recommended conditions for using DS.

Control surfaces are treated with a sterile drinking water also from the same calculation as the experimental ones.

Studies are carried out at a temperature of plus 18-20 ° C. When need assess the efficiency disinfection surfaces at elevated to plus 50 ° C or reduced to minus 30 ° C temperature (the tests are carried out in a thermo- or refrigerating cell).

Control of the effectiveness of disinfection of test surfaces is carried out in the following way: gauze napkin (size 5x5 cm), moistened at solution the relevant for of this

disinfectant of the neutralizer, thoroughly wipe
test-surface, then it is immersed in 10 ml of the same neutralizer,
located in test tubes with beads. Wash time for gauze wipes
10 min. with constant shaking. The washing liquid is sown
(for 2-3 cups of 0.1-0.2 ml per each) for solid differential-
diagnostic nutrient media.

Depending on the type of test microorganism, crops are grown
in a thermostat at a temperature of plus 37 ± 1 ° C. Accounting for results
within 1-2 days by counting the number of colonies grown,
then the density of contamination of 100 cm ^{2} of surface and%
disinfection, taking the number of colonies removed from the control
surfaces, for 100%.

The criterion of the effectiveness of disinfection of surfaces is not
less than 99.99% of death of test microorganisms; disinfection time
(min) with the contamination of S. aureus, E. coli, etc. - no more than 120 min.

1.1.3.6. Study of bactericidal effectiveness of DS,
intended for the disinfection of dishes

Depending on the purpose of the DS,
disinfection of tableware and kitchen, laboratory and from
excretions.

AT quality test objects at development of regimes
disinfection of the dining room and kitchen utensils use plates,
glasses, mugs of various materials (porcelain, faience, aluminum,
glass, plastic, dishes, covered with enamel) and cutlery - knives,
forks, spoons from various materials (stainless steel,
aluminum, plastic); laboratory glassware - subject and coverslips
glasses, pipettes, Petri dishes, plates for immunological

analysis, etc .; dishes from under the secretions - bedboards,
urinals, pots, spittoons, etc. or their test objects

imitating. As test microorganisms for contamination
tableware use S. aureus, E. coli, etc.

To simulate the contamination of laboratory glassware, 40%
inactivated serum; utensils from the vydeleny - 40%
fecal emulsion or sputum, contaminated test-
microorganisms (per 10 ml - 1 ml $2 \cdot 10^9$ billion suspended matter).

Before contamination by microorganisms, dishes and canteens
devices are mechanically cleaned - washed with water and soap and
brushing. The dishes are placed horizontally and a pipette is applied
Suspension of test microorganisms at the rate of 0.5 ml 2 billion suspensions per
The area in 100 sm^2 and evenly distribute it on a surface
glass spatula. Cutlery for contamination
immersed in a bacterial suspension for 1-2 minutes, leaving
their hands free.

The dishes are dried (until completely dry) at room temperature
temperature plus 18-20 ° C and relative humidity 50-60%,
then treated with a disinfectant solution.

For the development of disinfection regimes for dishes with residues
food before its contamination, the microbial suspension is mixed with oatmeal,
manna or other porridge, cooked on milk with butter
(to 10 g of porridge add 1 ml of 2 billion microbial suspension).

Processing of a dining room, tea, laboratory utensils and dining rooms
devices are carried out by immersion in a disinfectant solution.
The solutions are prepared in drinking water. The temperature of the test solution
plus 18-20 ° C. If necessary, study the effectiveness of solutions,
having a temperature of plus 50 ° C.

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Control is similarly contaminated dishes,
which is immersed in the same amount of drinking water.

The disinfectant solution must be completely and excessively
cover all dishes and appliances (at a rate of at least 2 liters per 1 set).

After certain time intervals (for example, 15, 30, 60 min.
etc.) are extracted from the disinfectant solution in one subject
(for example, a plate, glass, slide, knife, etc.) and sterile
gauze cloth (size 5x5 cm), moistened in a solution

a neutralizer corresponding to this DS; carefully wipe
the infected part of each item, immerse the napkin in 10 ml of this
same neutralizer, located in test tubes with beads. Waste time
gauze wipes 10 min. with constant shaking. After
washing gauze cloth is immersed in a suitable liquid
a nutrient medium. Washing liquid is sown for 2-3 cups
0.2-0.5 ml per each on solid nutrient media.

The crops are placed in a thermostat at a temperature of plus 37 ° C and
take into account after 2 days.

The disinfection time of the dishes is determined in the interval
from 15 to 240 minutes. depending on the type of test microorganism and the availability of
pollution.

The criterion of decontamination efficiency is not less than 100%.

The time of disinfection of tableware without food leftovers,
Contaminated: S. aureus, E. coli, etc. - not more than 60 min.

Time disinfection utensils from residues food,
contaminated S. aureus, E. coli, etc. - not more than 120 min.

Time disinfection laboratory utensils,
contaminated S. aureus, E. coli, etc. - not more than 120 min.

Time disinfection utensils from under excretions,
Contaminated E. coli, S. typhimurium - not more than 120 min.

1.1.3.7 Study of bactericidal effectiveness of DS,
intended for decontamination of secretions

Disinfectants facilities, intended for
disinfection of secretions, must have the ability
homogenize the organic substrate (feces, sputum).
Preparations that do not have this property for disinfection
excretions are not suitable.

A study of the activity of DS in the treatment of precipitates is carried out with
taking into account their consistency and the relationship with the disinfectant
solution or a dry preparation.

As test microorganisms in the development of regimes
disinfection of secretions using S. aureus, E. coli, etc.

Determination of the effectiveness of DS in urine treatment is carried out
as follows: take several tubes, pour in them
for 9 ml of urine, add 1 ml of suspension of the test microorganism,
containing $1 \cdot 10^9$ cfu / ml. Undiluted DS or its solutions
add to the urine in different ratios (equal, double, etc.).
After expiration of the exposure time (15, 30, 60, 90, 120 min.), Pipette
take 1 ml of the test mixture and transfer to a neutralizer in a volume of 9 ml, and
then from it 1 ml of the mixture into a tube with 5 ml of broth. After careful
mixing 1 ml is transferred to a second tube with broth, and then
make crops of 0.1 ml on solid nutrient media, both from the first,
and from the second test tubes. Petri dishes with crops are placed in a thermostat.

Control is similarly performed experiments with
adding to the urine is not a disinfectant solution, but drinking water.

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The DS is considered to be effective, providing a 100%
microorganisms in 6-8 experiments with coincident results.

Determination of the effectiveness of DS in the treatment of faeces: 20 g
Feces are ground in a mortar and add 80 ml of sterile drinking water
water. The resulting emulsion is filtered through a double layer of gauze,
poured into test tubes of 9 ml and add 1 ml of a suspension of test-
microorganisms containing $1 \cdot 10^9$ cfu / ml.

The prepared emulsion of feces is filled with equal or
with a double amount of disinfectant solution or
a different amount of a dry preparation. After contact with DS
produce seedings in the same way as in the disinfection of urine.
The results are taken into account after 2 days.

With positive results, experiments with large
number of decorated feces (200-250 g). To do this, place them
in a vessel and pour a disinfectant solution in an equal or
double the amount with respect to the weight of feces or fall asleep dry
drug. Then a small part of the fecal masses of the glass
The stick is mixed with the liquid, and the remaining mass is left in

the form of small lumps. At regular intervals
(30, 60, 90 and 120 minutes) separate the liquid part and
lumps.

The liquid part of the fecal masses is pipetted and produced
sowing the same way as urine. Dense parts of feces are taken up by a loop and
is lowered into 5 ml of nutrient medium by ripping them on the edge of the tube and
thoroughly mixed with broth; then transferred from this tube
1 ml of the mixture into a second tube, also containing 5 ml of broth. how
From the first, and from the second test tube, 0.1 ml of
Petri dishes with a dense nutrient medium.

Control serve similarly delivered experiments
with the addition of sterile water to the fecal emulsion instead of DS.

The effectiveness of the investigated DS is judged on the basis of
6-8 experiments with matching results. An effective DS is considered,
providing 100% doom test microorganisms at
disinfected material.

When determining the effectiveness of DS in the treatment of faecal-
urinary suspension in order to reduce microbial contamination
(conservation) as a test microorganism, a culture
E. coli (pc.1257). Fecal-urinary suspension is prepared from the calculation: 1 part
feces and 4 parts of urine. In the test tubes, 4.5 ml of fecal-
urinary suspension and add to each tube of 0.5 ml of a suspension of test-
culture, containing $1 \cdot 10^9$ cfu / ml. An undivided DS or its
solutions are added to the fecal-urinary suspension in various
relations. After the exposure time from each sample
0.5 ml of liquid is taken and transferred to test tubes with 4.5 ml
casein broth. This ensures neutralization of the DS. After a series of
Serial dilutions from each tube are seeded into cups
Petri with nutrient agar (meat-peptone, Endo). results
The experiment is taken into account after 48 hours of incubation at plus 37 ° C. AT
control samples instead of the drug used sterile
drinking water.

Effective considered DS, which provides a reduction in microbial

contamination of the faeces-urinary suspension by at least 99.9% and not more than 120 minutes.

The criterion for the effectiveness of disinfection of the precipitates is 100% death of test microorganisms.

The time for disinfection of secretions, contaminated test-microorganisms S.aureus, E.coli, etc. - no more than 6 hours.

1.1.3.8 Study of bactericidal effectiveness of DS, intended for air disinfection

The aim of the study is to determine the effectiveness of chemical DS and development of their application modes for disinfection of air in the premises.

Chemical DS are used for air disinfection in premises in the form of aerosols or vapors of DS solutions, as well as gases.

When investigating the effectiveness of air disinfection chemical S.Asureus is used as test microorganisms.

Studies are carried out in test chambers with a volume of 1-2 m^3 . Preliminary, the inner surface of the chamber is washed with a solution The detergent is then rinsed off with a tap water and include a bactericidal UV-irradiator. In the center of the camera have a disinfected fan, performance 15-25 m^3 / h, the purpose of which is to prevent rapid subsidence microorganisms.

The room in which the test chamber is located should be equipped with a recycling type UV irradiator that functions throughout the experiment. Before starting work the researcher puts on a robe, rubber gloves and a mask.

When determining the effectiveness of DS intended for disinfection of air, use 2 methods: sedimentation and aspirating.

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Sedimentation method.

To conduct studies using the sedimentation method, the chamber should have a window with a door of 20x20 cm for a chamber of petri dishes.

The melted agar with the added neutralizer is poured over Petri dishes (do not dry the nutrient medium).

Before the spraying begins, the suspensions of the test microorganism are taken A test to control the dissemination of air in the chamber, for which purpose The open Petri dish with the nutrient medium is placed for 30 minutes. at camera.

Then, the suspension of the test microorganism is sprayed into the chamber sufficient to create a concentration chamber in the air microorganisms $2,1x10^4$ CFU / m^3 .

Aerosol is created with the help of spray equipment, which ensures the formation in the air of not less than 80% of the particles with dispersion of 10 + 5 μm, and include a fan. For control degree of air contamination, an open Petri dish with a nutritious the medium is placed in the chamber for 10 minutes.

Then a solution of the test substance is sprayed into the chamber and through certain intervals of time check the dissemination of air.

Aspiration method.

This method is based on aspirating air through a liquid. When using the aspiration method to assess the effectiveness DS, intended for air disinfection, is necessary the following equipment:

– blower with a capacity of 15-25 l / min .;

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– sterile Drexel flasks with 50 ml of sterile

tap water at the rate of 2 pcs. for 1 trial. Preliminary in
Sterile water is injected with an appropriate neutralizer;

– α tube with a diameter of 8-10 mm, a length of 50-60 cm, which is introduced in

chamber for sampling air in the center of the chamber;

– sterile rubber hoses connecting Drexel's flasks

(successively one after another) and then with a blower.

Preparing the camera for the experiment is carried out as indicated

higher. For the study, 50 liters of air (volume of the sample) is taken.

The order of sampling is as follows:

– control of air contamination prior to spraying

suspensions of test microorganisms;

– control of air contamination after spraying the suspension

test-microorganism;

– control of decontamination efficiency - sampling through

every 5-10 minutes, depending on the expected effectiveness of the DS.

After sampling, the liquid from the 2 Drexel bottles is mixed

and 1-2 ml are added to a sterile Petri dish; then poured

melted agar. The crops are placed in a thermostat. After incubation

count the number of colonies that have grown and count

seeding of 1 m^3 of air.

For example:

$$X = \frac{10 \cdot 0.05 \cdot 1000}{50} = 10$$

X - CFU / m^3

10 - the number of colonies grown on a petri dish;

0,05 - volume of sterile drinking water, l;

1000 = 1 m3 of the chamber air, l;

50 - volume of air sample, l.

Criterion of air disinfection efficiency in accordance with

with a category of a premise,% (not less): I - 99,9; II - 99,0; III - 95,0;

IV - 90,0; V - 85.0

1.1.3.9. A study of the effectiveness of DS intended for

disinfection of drinking water and swimming pool water

Methods apply to tests of chemical DS for disinfection of individual and group drinking water supplies and water swimming pools. Disinfected drinking water must be comply with the requirements of national regulations.

SanPiN 2.1.4.1175-02 "Hygienic quality requirements water of decentralized water supply. Sanitary protection sources ", GOST 27283-87, and the water of swimming pools - requirements SanPiN 2.1.2.1188-03 "Swimming pools. Hygienic requirements for the device, operation and quality of water. Quality control".

DS for disinfection of drinking water must provide death in water of test microorganisms at their initial concentration:

bacteria that do not form spores - 10^5 - 10^6 cfu / l;

viruses - 10^5 - 10^6 UE / l;

bacteria in spore form - not less than 10^5 - 10^6 spores / l (with necessity - depending on the purpose of the facility, see 5.8.).

DS for water disinfection of swimming pools should To ensure the death of test microorganisms in water at the initial concentration:

in the development of regimes for the continuous disinfection of water in presence of visitors:

bacteria that do not form spores - 10^2 - 10^3 cfu / l;

viruses - 10^2 - 10^3 UE / l.

when developing regimes for disinfection of water during long break in the pool (more than 2 hours):

bacteria that do not form spores - 10^5 - 10^6 cfu / l;

viruses - 10^5 - 10^6 UE / l.

Test microorganisms.

As test microorganisms in the study of means disinfection of drinking water use E.coli (strain 1257), RNA-containing coliphage MS2.

As test microorganisms in the study of means
Disinfection of swimming pool water using crops
E. coli (strain 1257), S. aureus (strain 906), and RNA-containing coliphage
MS2.

Nutrient media, methods of cultivation and preparation
for experiments of test cultures of bacteria that do not form spores,
are given in p. 1.1., 1.3., 1.7.

Kolifag MS2 (strain VKPM-3254) and strain of cell culture-
The host of E.coli K12 F + StrR is obtained in the All-Russian Collection
industrial microorganisms (VKPM) of GNII "Genetics", address:
113545, Moscow, 1-st Road travel, 1.

Studies are conducted in accordance with the Methodological
"Sanitary-microbiological analysis of drinking water"
MUK 4.2.1018-01, Ministry of Health of Russia, Moscow, 2001.

Nutrient media and reagents for coliphage MS2 and E. coli K12 F + Str^R:

Dry nutrient broth

Dry nutrient agar	TU 42-14-33-75
Streptomycin is sterile	
Technical chloroform	GOST 20015-76

Preparation of nutrient media for coliphage MS2 and E. coli K12
F + StrR.

Nutrient broth is prepared from a dry preparation of industrial
production by the method indicated on the label; nourishing broth
(tenfold) for coliphages - by increasing the weight 10 times
the dry preparation indicated on the label; nutrient agar - from dry
preparation of industrial production by the method indicated on
label; nutrient agar double concentration to determine
coliphages by direct method - by increasing the amount of dry
of the drug in 2 times from the prescription.

Nutrient agar is not allowed to stand in a molten agar
more than 8 hours. The remaining unused agar is repeated
It can not be melted.

Semi-liquid nutrient agar is prepared as follows:

dry nutrient broth (15 g) and microbiological agar (3 g)
dissolve when heated in 1000 ml of distilled water. Bring
pH to 7,0 - 7,2, pour into tubes and sterilize by autoclaving
at a temperature of 121 ° C for 15 minutes.

Nutrient agar with streptomycin is prepared from the calculation of
of the content of 100 μg of streptomycin per 1 ml of nutrient agar,
prepared according to the standard prescription. Sterile on sterile
distilled water is prepared a solution of streptomycin in concentration
10 mg per ml. In the ready nutrient agar, measured by volume and
cooled to a temperature of 45-49 ° C, the cooked

Sterile streptomycin solution at the rate of 0.1 ml per 10 ml
nutrient agar. Pour into test tubes for cooking
bevelled agar. Repeated melting of the nutrient medium with
streptomycin is prohibited.

Conducting cultures of colifag MS2 and culture of E. coli host cells
K12 F + StrR. The process of conducting colophage cultures of MS2 and E. coli K12 F +
StrR includes:

restoration of lyophilized cultures of coliphage MS2 and
E. coli K12 F + StrR;

creation of stocks of colonies of colifag MS2 and E. coli K12 F + StrR for
target use.

For restoration of lyophilized culture
E. coli K12 F + StrR the drawn-out end of the ampoule with lyophilized
culture heated over the flame of the burner. Wet end
sterile cotton swab touching the heated part, in
resulting in cracks. The end of the ampoule is covered
a three-layer gauze cloth moistened with 70% ethyl alcohol and
well wrung out, and break off tweezers. After opening the ampoule
remains covered with the same napkin for 1-2 minutes. Then a napkin
carefully removed and, together with the remains of the glass, immersed in
disinfectant. 0.5 ml of nutrient broth is added to the ampoule.
rehydration. Ampoule contents are mixed, transferred
with a sterile Pasteur pipette or syringe into a test tube with

nutrient broth and incubated at 37 ± 1 ° C in
within 18-24 hours.

After incubation from the nutrient broth, sow a loop on
chopped nutrient agar according to TU 42-14-33-75, containing

streptomycin. The cultures are incubated at 37 ± 1 ° C for
18-24 hours

Culture on sloping nutrient agar is checked for
The ability to lyse specific MS2 coliphage, as well as
contamination with phage. With satisfactory results of these
The culture is considered suitable for conducting research with
colitis with MS2.

To create a working crop stock E.coli K12 F + StrR
culture from sloping nutritional agar with streptomycin
inoculated with a stab in a column with a semi-liquid nutrient agar. Crops
are incubated at a temperature of 37 ± 1 ° C for 18-24 hours. If available
growth tubes are closed with rubber (silicone) stoppers and
put into storage at a temperature of 4-8 ° C. Stock replenishment
Working culture is carried out after 3 months.

At all stages of the study, a bacterial suspension
culture E. coli K12 F + StrR, prepared as follows.
Culture grown in a test tube with chopped nutrient agar
with streptomycin, wash off from the jamb 5 ml sterile
physiological saline and a turbidity standard prepare the slurry in
concentration of 109 bacterial cells in 1 ml. Allowed
use of the 4-hour E. coli broth culture obtained by
growing in a thermostat at a temperature of 37 ± 1 ° C. Concentration
109 bacterial cells of E. coli are contained in 2 ml.

To restore the lyophilized culture of colifag MS2
the drawn end of the ampoule with the lyophilized culture is heated
above the flame of the burner. The wet end of a sterile cotton swab
touch the heated part, resulting in cracks.
The end of the ampoule is covered with a three-layer gauze cloth moistened with

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70% ethyl alcohol and well wrung out, and break off with tweezers.
After opening, the ampoule remains covered with the same tissue for
1-2 min. Then the napkin is carefully removed and, together with the rest
the glass is immersed in the disinfectant. In the ampoule, 0.5 ml of nutritious
broth for rehydration.

To create stocks of culture of colitis, MS2 is used
working culture of E. coli K12 F + StrR host cells stored on
semi-liquid agar, which is inoculated into a test tube with 10 ml of nutrient
broth. The cultures are incubated at 37 ± 1 ° C for
18-24 hours

After incubating 0.1 ml of the resulting broth culture of E. coli
K12 F + StrR is resuspended in 3-4 tubes with 10 ml of nutrient
broth and placed in a thermostat at a temperature of 37 ± 1 ° C. After 2 hours
incubation, a rehydrated phage culture was introduced into each tube
MS2, the incubation is continued until 18-24 hours. After incubation in a test tube
add 1 ml of chloroform, tightly seal, intensively
shake and place in the refrigerator.

The titer of the obtained colophage MS2 culture is determined as follows
way. Prepare 7-8 tenfold dilutions of colifag culture
MS2. 1 ml from each dilution is added to Petri dishes and poured
mixture of nutrient agar and a suspension of E. coli K12 F + StrR culture from
Calculation of 1 ml of suspension per 100 ml of agar. Crops are incubated at
temperature 37 ± 1 ° C. Test tubes with dilution are clogged
rubber (silicone) stoppers and stored until
results at a temperature of 4 ± 2 ° C to obtain working suspensions
phage.

After 18-24 hours of incubation, the number of plaques on the plates is counted.
Cups are accounted for, on which growth of 30-100 negative

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colonies of phage. For use, a culture with a title of

less than 107.

When receiving a titer, less than 107 phages can be multiplied. For this it is necessary to repeat the described procedure.

Technique of statement of experiments.

The source water is tap water or Natural water (well, river), the quality of which correspond to purpose of the tested disinfectant facilities. The tap water is dechlorinated before use heating at a temperature of 50-60 ° C., followed by maintaining for one day. In samples of natural water determine the total microbial number and common coliform bacteria.

The initial water is poured into a container with a lower tube of 5-10 L and contaminated with cultures of test microorganisms.

After applying the calculated dose of microorganisms, water carefully mix and sample for concentration initial contamination of water by microorganisms.

Concentration source infection water bacteria is defined as follows. 1-3 water samples are taken from the vessel (depending on the volume of the container) for 3-5 ml. From each sample do 3-4 consecutive tenfold dilutions in sterile physiological solution or sterile tap water, in which are then determined by the number of bacteria in 1 ml of water sample by membrane filtration.

Essence method is at concentrating microorganisms from a certain volume of analyzed water by filtering through membrane filters, growing crops under temperature 37 ± 1 ° C on dense nutrient medium and in counting

the number of bacteria per unit volume of water. The membranes filters for microbiological purposes with a pore diameter of not more than 0.45 μm and a disc size of 35 or 47 mm or other filter media membranes with a similar filtration capacity, having certificate of quality. Membrane filters are prepared for analysis in according to the manufacturer's instructions.

The filtration of water is carried out using a device for membrane filtration under vacuum with a filter surface diameter of 35 or 47 mm with a device for creating a vacuum of 0.5-1.0 atm. Funnel and the table of the device before the analysis of water is sterilized in a steam or air sterilizer. On the lower part of the device (table) flamed tweezers sterile membrane filter; press it with the upper part of the device (glass, funnel); fixed by a device provided by the device design, pour in accordance with the rules of sterility required volume water and create a vacuum in the receiving vessel. Filter first smaller, then large volumes of water through a single filter device, changing filters each time. After filtering is complete the glass (funnel) is removed, the filter is gently lifted off the edge flambied tweezers while maintaining vacuum for removal excess moisture on the underside of the filter, and then transfer it, not turning over, on a dense nutrient medium in a Petri dish so, so that there is no air bubble between the medium and the filter.

When determining the number of E. coli bacteria, a nutrient agar Endo, S.aureus - casein or meat-peptone agar. Under Each filter on the underside of the bottom of the Petri dish is made An inscription indicating the volume of filtered water, the date of sowing and sample numbers. Crops from E. coli, S. aureus are incubated at a temperature of

37 ± 1 ° C for 24-48 hours. At the end of the incubation take into account The number of colonies grown on filters is determined concentration of test microorganisms in 1 liter of water according to the formula:

$$C = \frac{TO}{V} N \bullet 1000,$$

where C is the number of test microorganisms contained in 1 liter water;

K is the multiplicity of the dilution;

V - sown volume in ml;

N is the arithmetic mean of the number of colonies grown on membrane filters for seeding the same dilutions.

The result of the analysis in determining the number of bacteria expresses the number of colony forming units (CFU) per 1 liter of water.

The concentration of initial water contamination with colitis MS2 is defined as follows. At the initial concentration coliphage at level 10^5 -10^6 pfu / l, a sample of water volume of 5-10 ml. Prepare 3-4 consecutive tenfold dilution in sterile saline or sterile tap water. 1 ml from each dilution is added to the cups Petri and filled with a mixture of nutrient agar and suspension of E. coli culture K12 F + StrR at the rate of 1 ml of suspension per 100 ml of agar. Crops are incubated at a temperature of 37 ± 1 ° C. After 18-24 hours incubation is counted the number of plaques on the plates. Cups are subject to accounting, on which there is an increase in 30-100 negative colonies of the phage. Count the number negative colonies on 2-3 cups with the most characteristic and clear growth. According to these data, the average number of colonies is determined, which multiply by the largest dilution.

With an initial coliphage concentration of 10^2 -10^3 pfu / l of a 150-200 ml sample is taken. Prepare a tenfold dilution in a sterile physiological solution or sterile tap water. Colitis is defined in 1 ml of 10-fold dilution, as well as in 1, 10 and 100 ml of the original contaminated water.

Sowing 1 ml of a ten-fold dilution, as well as 1 ml of the original water is carried out both at the initial contamination of water at the level of 10^5-10^6 pfu / l.

Inoculation with 10 ml of the original water is carried out as follows. 10 ml of the original water is introduced into the nutrient agar of a double concentration, melted and cooled to 45-49 ° C, washed with E. coli K12 F + StrR from the calculation of 2 ml of flush (or 4 ml of a 4-hour broth culture) for every 100 ml of agar, mix. Cups with crops leave at room temperature until freezing. Cups with the frozen agar is placed in a bottom up in a thermostat and incubated

at a temperature of 37 ± 1 ° C for 18 ± 2 hours.

Inoculation with 100 ml of the original water is carried out as follows. In a nutrient agar double concentration, melted and cooled to 45-49 ° C, washed with E. coli K12 F + StrR from the calculation 2 ml of flush (or 4 ml of a 4-hour broth culture) for every 100 ml agar, mix. The investigated 100 ml of water is poured into 20 ml in large test tubes, heated to plus 35-44 ° C and immediately (no more than than in 5 minutes. after reaching the required temperature) is poured in 5 Petri dishes and immediately add to each cup 20 ml of the mixture agar with E. coli K12 F + StrR culture, gently agitate.

To control the culture of E. coli K12 F + StrR in a single Petri dish add 20 ml of sterile tap water,

heated to 35-44 ° C, poured 20 ml of the prepared agar with E. coli K12 F + StrR and gently stir.

The plates with crops are left at room temperature up to congealing. Then the cups with the frozen agar are placed upside down in thermostat and incubated at a temperature of 37 ± 1 ° C for 18 ± 2 hours. The results are taken into account by counting and summarizing the plaques, grown on 5 Petri dishes. The results are expressed in plaque forming units (PFU) per 100 ml of water sample. AT the control plate of the plaque should be absent.

Based on the results of determining the number of particles of coliphage in 1, 10 and 100 ml of the initial water, the initial water contamination with coliphage and express it in pfu / l.

For disinfection of water DS are introduced into a container with an infected water in the necessary concentrations and mix thoroughly. Across specified intervals of time under the conditions of sterility Samples of water of 1 L volume are taken into sterile bottles with added in them a neutralizer selected according to the formulation disinfectant, and determine the microbiological indicators of disinfected water. Water samples should be are investigated not later than 1 hour after their selection. On condition storage of samples in the refrigerator at a temperature of 1-5 ° C is allowed carry out the analysis no later than 6 hours after sampling.

The number of bacterial cultures of S. aureus, E. coli in disinfected water is determined by the membrane filtration method.

At the initial stages of water disinfection, less than two water samples, differing by volume in 10 times and selected so that on one of the filters no more

300 colonies. For one cup, you can put 3-4 filters with the condition, so that filters do not touch each other.

When analyzing water at the final stages of disinfection, investigate volume not less than 1 liter, filtering this quantity not less than through 3-4 filters.

The crops are incubated as indicated in 5.2. Take into account the number grown colonies on filters, the result of the analysis is expressed by the number bacteria in 1 liter of water.

Identification of total and thermotolerant coliforms bacteria.

The common coliform bacteria (OCD) are Gram-negative, oxidase-negative, non-controversial Sticks capable of growing on differential lactose media, fermenting lactose to acid, aldehyde and gas at a temperature of plus 37 ± 1 ° C for 24-48 hours.

Thermotolerant coliforms (TKB) are included in the number common coliform bacteria, possess all of their characteristics and, in addition to Moreover, they are able to ferment lactose to acid, aldehyde and gas at a temperature of 44 ± 0.5 ° C for 24 hours.

If there is no growth on the filters or if only filmy, spongy, moldy, transparent and diffuse colonies after 18-24 hours they give a negative answer.

With the growth of the colonies on the filters, characteristic of intestinal sticks (dark red with metallic luster and without it, red, pink mucous, pink with a dark center, colorless, with imprint on the back of the filter), perform an oxidase test, for which the membrane filter is lifted upwards by a colony into a circle

filter paper, wetted with reagent to determine
oxidase activity.

Given the bactericidal activity of the reagents for determining
oxidase activity, the membrane filter immediately after the manifestation
The reaction should be transferred back to the Endo medium.

The presence of active oxidase (a change in colony color on blue-
purple) in all colonies, with the exception of those that are not characteristic of the OKB and
TKB, allows to give a negative answer and to finish the analysis through
18-24 hours

If colonies that do not have oxidase activity have grown,
a few colonies of each type are prepared by smears, stained by
Gram and microscopy.

Absence in smears of gram-negative, non-controversial
sticks, allows to give a negative answer and finish the analysis through
18-24 hours

Red and dark red colonies with metallic luster and without
(lactose-positive), formed by gram-negative
Chicks that do not have oxidase activity are counted and
are referred to general and thermotolerant coliform bacteria.

The result of the analysis is expressed by the number of bacteria in 1 liter of water.

Determination of the number of particles of colifag MS2 in treated water in
samples with a volume of 1 liter are carried out by the enrichment method (Kornilova NM,
1988). For this purpose, a sample of disinfected water of 1 liter is poured into
sterile bottles of 500, 200, 100, 50 and 20 ml, add 10%
a ten-fold nutrient broth for coliphages and a suspension
of the daily culture of E. coli K12 F + StrR. After incubation with
temperature 37 ± 1 ° C for 18-24 h, the liquid is poured into test tubes,
are shaken with chloroform to eliminate bacterial flora and

determine the number of particles of the coliphage MS2. To do this, 1 ml of each the test tubes are poured into Petri dishes and poured with a mixture of nutrient agar and suspension of E. coli K12 F + StrR culture at the rate of 1 ml of suspension per 100 ml agar. The cultures are incubated at a temperature of 37 ± 1 ° C. After 18-24 hours Incubation counts the number of plaques on the plates. Depending on the In which volume the growth of coliphage was detected, the most the probable number of phage particles in 1 liter of water, using Table. 1.3.

Table 1.3.

Calculation of the number of phage particles in determining titer of phage in 1 liter of water by enrichment

The presence of phage after incubation in water samples of volume (ml)					Number of PFU in 1 liter of water
500	200	100	50	20	
—	—	—	—	—	0
+	—	—	—	—	2
+	+	—	—	—	5
+	+	+	—	—	10
+	+	+	+	—	20
+	+	+	+	+	≥ 50

Treatment and assessment of the results microbiological research. Statistical treatment the results microbiological analysis to assess the effectiveness of methods and water disinfection is designed to eliminate accidental errors, estimate the deviation of the test results from the actual and give the desired results with a given probability.

Random errors in bacteriological analysis, as a rule, are distributed according to the normal law, so in the process statistical processing the results experimental research is recommended to use in determining concentration of contamination of the source water and at intermediate stages

processing - the arithmetic mean (x), and in assessing the effectiveness water disinfection at the final stage - median value (Me).

The number of trials n required for a valid estimate The results of microbiological studies are determined by

formula:

$$n = \left(\frac{\sigma t_{p\,2}}{I_p}\right),$$

where σ is the quadratic deviation;

Ip is the maximum permissible deviation from the mean,

estimated with probability p = 0.99;

tp is a coefficient that depends on the number of experiments (at least 10), on which determined the value of σ.

If σ is determined from the data of 16 experiments, then $t_{0.99} = 2.7$.

Number of samples for assessing the content of microorganisms in

Disinfected water must be at least 16.

Evaluation of the results of bacteriological analyzes is carried out for

given a probability of 0.99, respectively, for the same value

determine the confidence interval of the arithmetic mean and

medians.

The confidence interval of the arithmetic mean is determined by

given the value of the quadratic deviation σ and the mean error σ x.

The value of the quadratic deviation is calculated by the formula:

$$\sigma = \sqrt{\frac{\Sigma (x - x)_2}{n - 1}},$$

where Σ (x - x) 2 is the sum of the squared deviations of the results

individual measurements from the arithmetic mean, and n is the number

individual measurements.

The average error is calculated by the formula:

$$\sigma_x = \frac{\sigma}{\sqrt{n}}$$

The probability of 0.99 corresponds to the confidence interval </s>,

calculated by formula

$$I_{0.99} = \pm 2.7\,\sigma_x$$

The median (median) confidence interval for

The required probability level of 0.99 is determined depending on the number

conducted experiments according to Table 1.4., in which the experimental numbers are indicated, the results of which are taken into account as boundary values confidential result of the median.

Table 1.4.

Boundary values of the median confidence interval

Number of experiments	Lower border	Upper border	Number of experiments	Lower border	Upper border
7th	-	-	27th	7th	21
8	1	8	28	7th	22
9	1	9	29	8	22
10	1	10	thirty	8	23
eleven	1	eleven	31	8	24
12	2	eleven	32	9	24
13	2	12	33	9	25
14	2	13	34	10	25
15	3	13	35	10	26th
16	3	14	36	10	27th
17th	3	15	37	eleven	27th
18	4	15	38	eleven	28
19	4	16	39	12	28
20	4	17th	40	12	29
21	5	17th	41	12	thirty
22	5	18	42	13	thirty
23	5	19	43	13	31
24	6th	19	44	14	31
25	6th	20	45	14	32
26th	7th	20	46	14	33
			47	15	33
			48	15	34
			49	16	34
			50	16	35

To use the table. 5.4. it is necessary that the results of the experiments were arranged and numbered in the order of increasing of their magnitudes.

An indicator of the effectiveness of DS intended for disinfection of water, is a 100% reduction in water contamination, contaminated test microorganisms, that is, the absence of test-microorganisms in 1 liter of disinfected water.

1.2 Methods of studying and evaluating tuberculocidal activity disinfectants

1.2.1 General

To study and evaluate tuberculocidal activity of DS in The following microorganisms are used as test microorganisms: Mycobacterium terrae (DSM 43227), Mycobacterium B5, Mycobacterium tuberculosis, Musobasteriitis smegmatis sir 7326, Musobasteriitis avium ATCC 15769 (DSM 44157).

Research tuberculocidal activity of substances, DS and
The effectiveness of the DS regimes includes:

selection and preparation of test-microbial cultures for study
tuberculocidal activity of DS and substances;

ensuring the standard of research conditions
tuberculocidal activity of DS and substances;

methods of research and evaluation of tuberculocidal
activity of DS and substances in vitro (suspension method, method
batistovye (bjazevyh) test objects);

methods of studying tuberculocidal efficacy of DS with
use of artificially contaminated test-
Mycobacteria of various test objects for the purpose of developing

regimes of disinfection of certain objects in relation to
causative agent of tuberculosis (surfaces in rooms, transport,
sanitary equipment, furniture, utensils, linen, clothes,
care products, medical devices, tools and
other medical products, etc.);

methods of research of tuberculocidal efficacy of DS in
practical conditions.

1.2.1.1 Test-microorganisms for studying tuberculocidal activity of disinfectants and their substances.

Requirements for test microorganisms

Research and evaluation of the tuberculocidal and mycobactericidal (in
non-pathogenic mycobacteria) of the activity of the DS, proposed
for use on the territory of Russia, are carried out using
test microorganisms:

agar culture of Mycobacterium B5 for evaluation of the efficacy
and development of tuberculocidal regimens of chamber disinfection
different objects;

agar culture of Mycobacterium terrae (DSM 43227) for evaluation
activity of DS and development of modes of their application at
disinfection of facilities in relation to the causative agent of tuberculosis and

mycobacteriosis;

agar culture of Mycobacterium tuberculosis for confirmation effectiveness of developed regimes of application of DS in relation to causative agents of tuberculosis and mycobacteriosis in practical conditions.

Test-mycobacteria should have typical morphological, cultural, biochemical, tinctorial and enzymatic properties inherent in this strain (see Appendix 1), and possess

standard resistance to the reference DS and temperature. Culture Mycobacterium B5 should be resistant to a temperature of 60 ° C for 60 min. Indicators of sustainability that must be met culture of Mycobacterium terrae are given in Table 1.5.

Table 1.5.

Stability of Mycobacterium terrae to reference DS

DS	Concentration of solution,%	Time of death Mycobacterium terrae, min.
Chloramine B (28.0% for active chlorine)	5.0 *	120
Glutaraldehyde	0.5	60
Hydrogen peroxide	4.0	60

Note: * The concentration of the drug is indicated

Preparation and storage of the initial working culture of test-mycobacteria

To grow cultures, test-mycobacteria are used dense nutrient media. List and procedure of preparation nutritive environments for cultivation test-mycobacteria, intended for the study and evaluation of tuberculocidal activity DS and their substances are given in Appendix 2. In contrast to Nutrient media recommended in the standard NEN-EN 14476, Use of the nutrient media provided in the application allows receive the result of evaluation of tuberculocidal activity of DS not for 21 days, but for 10-14 days.

To obtain a culture of a test-mycobacterial strain, an ampoule with lyophilized museum culture of this strain is opened and A sterile pipette into the ampoule is poured into 1.0 ml of sterile saline and left for 30 minutes at room temperature to obtain a slurry. The suspension,

prepared from a museum test culture, dissipate in 0.1 ml of Levinstein-Jensen infusion medium or

"New" (only 10 tubes). The crops are incubated in a thermostat at 37 ± 1 ° C for 14-21 days.

The resulting biomass is removed with a platinum spatula or A glass rod from the surface of the Levenstein-Jensen or "New" from all tubes and placed in a test tube with 10 ml broth Middlebrook 7N9 with 10% ADC growth additive, mixture homogenize, the volume of the resulting suspension is adjusted to 100 ml broth Middlebrook 7H9 and 0.5 ml of the suspension is introduced into cryovials.

The suspension is frozen at minus 70 ° C or stored in a domestic refrigerator at minus 20 ° C. The shelf life of the crop in such conditions - no more than 5 years.

The culture of test-Mycobacterium is used to produce agar culture test-Mycobacterium (first passage) and preparation of its working suspension of test-mycobacteria used for testing DS.

1.2.1.2 Method of preparation of a suspension of test microorganisms. Determination of the biological concentration of test microorganisms in suspensions

To obtain the first passage of culture of the test strain Mycobacterium used in the evaluation of DS is necessary for the amount stored at minus 70 ° C or minus 20 ° C of cryo-tubes (2 pieces per 1 study) with this strain of mycobacterium is thawed at room temperature, transfer 0.1 ml of the contents in tubes with beveled Leuvenstein-Jensen nutrient medium or "Novaya" and incubate

in a thermostat at 37 ± 1 ° C for 14-21 days. Growing on a dense
culture medium is used in test tubes for
preparation of a working suspension of test-mycobacteria of this strain.

Cultures of test microorganisms are subjected to control of their
quality. In particular, just before using the crops
for research purposes it is necessary to make sure that the test-
strains grown on a nutrient medium are not contaminated by extraneous
microflora. To assess the growth of cultures of mycobacteria of test strains
visually scan each tube and take into account the nature and
massiveness of growth, change in the color of the nutrient medium. Conducted
microscopy of the smear of grown cultures by the method of coloring according to Tsil-
Nielsen (M.terrae are short straight sticks
crimson-red color, located in the smear parallel to each other
friend, like a stockade. Size of mycobacterial cells
0.2-0.6x1-10 μm).

The working suspension of the test-mycobacterial culture is prepared from test-
strain of the first and / or second passages, grown on dense
nutrient medium. Further subculturing is unacceptable!

To prepare a working suspension, the culture of mycobacteria
are removed with a platinum spatula or a glass rod with a dense
and placed in a thick-walled glass vial.
The microbial biomass is thoroughly homogenized, gradually adding
dropwise sterile saline. Thick initial
the bacterial suspension is allowed to stand for 15 minutes. for deposition
inhomogeneous conglomerates and particles. The received
the supernatant is taken with a Pasteur pipette, transferred to
A sterile tube, the diameter of which corresponds to the diameter
tubes with an optical turbidity standard (turbidity standard

production of FGBU "NTSESMP" of the Ministry of Health of Russia, the standard of turbidity
by McFarland) and standardized according to the optical turbidity standard,
corresponding to $1 \cdot 10^9$ microbial bodies in 1 ml), adding a sterile
distilled water.

A suspension containing such an amount of living microbes, with contamination by her coarse (cambric) test-objects, test-surfaces and other provides the required (of the order $1 \cdot 10^{5}$-$1 \cdot 10^{6}$ CFU / cm^2) levels of their seeding with living cells of test-microbe.

However, due to the fact that mycobacteria can die off in result of storage or for other reasons, and dead the microorganisms present in the suspension will, like the living, give clouding suspension, is necessary realize bacteriological control of the actual number of living cells in prepared suspension to, if necessary, add adjustments and provide the required levels of contamination test-objects viable mycobacteria.

Determination of the biological concentration of the test microbe in a working suspension

From a suspension prepared according to the optical standard turbidity №10 (10^9 cfu / ml), do as shown in the scheme 1.2., dilutions with 10-fold steps to 10^3 microbial cells per 1 ml (seeding 0.1 ml of the suspension from this dilution to a dense nutrient medium allows you to make a fairly accurate calculation of those grown on the environment colonies of mycobacteria, the number of which will be in within 100 units).

Scheme 1.2.

Conducting an experiment to control the number of live Mycobacteria in the working suspension of test-mycobacteria used for evaluation of tuberculocidal activity of DS

Dilutions are prepared as follows:

1 ml 1 ml 1 ml 1 ml 1 ml 1 ml Test tubes with dense nutrient medium to 0.1 ml

Suspension by 1 2 3 4 5 6 Test tubes with 9 ml

to the turbidity standard №10 (10 9 cfu / ml) № breeding sterile ds.

The first dilution corresponds to 10 8 CFU / ml, and the sixth - 10 3 cfu / ml. From this dilution, a 0.1 ml on 5 tubes with medium of Levenshtein-Jensen or "New" incubate in a thermostat at 37 ° C for 14-21 days.

Count the number of colonies grown on medium in vitro, the average of 5 is calculated and the quantity viable cells in the initial suspension, considering the coefficient breeding. The number of viable cells in the working suspension should be 10 9 cfu / ml.

Sowing the suspension in tubes with sloping dense nutritious environment can significantly save the flow of the environment and avoid drying and cracking of the medium observed when using Petri dishes with a dense nutrient medium.

Calculation of the number of viable bacterial cells in The prepared initial suspension is carried out according to the following formula:

X = A x 10 7 , where

X is the number of viable bacterial cells in 1 ml a prepared suspension;

A is the average number of colony forming units (CFU), grown on 5 Petri dishes;

107 - conversion factor, taking into account the degree of dilution suspension (106) and volume used for inoculation (0.1 ml).

For example:

the growth of mycobacteria in the first sample was 70 cfu, in the second - 130 cfu, in the third - 99 cfu, in the fourth - 115 cfu, in the fifth - 97 cfu.

Then the average number of colony forming units (CFU), grown on 5 test tubes, will be:

A = (70 + 130 + 99 + 115 + 97): 5 = 102

X = 102 x 107 = 109 microbial bodies in 1 ml, which corresponds to optical turbidity standard No. 10 (1 billion microbial bodies (109) in 1 ml.

1.2.1.3. Determination of resistance of test microorganisms to standard DS

Determination of resistance of test-mycobacteria to effects temperature.

In a sterile glass beaker with a volume of 100 ml, pour 50 ml sterile distilled water and placed in a water bath.

Next to it in a water bath is placed a glass beaker with a volume of 100 ml with 50 ml of distilled water and with a thermometer placed in it, allowing to measure the temperature up to 100 ° C. Turn on the water bath, bring the water temperature in the glass to 60 ° C and support this temperature throughout the experiment.

In sterile tubes, 5 ml of sterile drinking water at room temperature (the number of tubes corresponds to number of samples taken in the experiment). Based on the number of samples, test tubes with sloping dense nutrient medium Levenshtein-Jensen or "New". Prepare and contaminate the test-microorganisms, batistovye test objects (clause 1.1.2.2.). If in experiments use previously prepared and stored in the refrigerator contaminated test bacilli, then in advance are removed from the refrigerator so that they can buy a room temperature (18-20 ° C).

Count in the Petri dish the amount required for the experiment batistovyh test objects (2 for each exposure), contaminated test-mycobacteria, capture test objects Sterile tweezers all at once and dip them into a glass with a sterile drinking water heated in a water bath to 60 ° C. Easy Wiggle the tanks to achieve full wetting of the test objects. At the time of wetting all test objects, time is noted.

Every 15 minutes. Sterile tweezers extract 2 test- and drop them into a test tube with 5 ml of sterile drinking water at temperature of 20 ± 2 ° C to neutralize the effect of temperature

factor a. After 5 minutes each test object is placed on the surface cut in a test tube of a dense nutrient medium.

To control two contaminated test-mycobacteria test-objects are immersed in sterile drinking water at a temperature of 20 ± 2 ° C for the maximum exposure time, then (as well as experienced test-objects) they are placed on the surface of a canted in the test tube dense nutrient medium.

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Experimental and control sowings are placed in a thermostat at temperature 37 ± 1 ° C; The presence of growth of test cultures is checked through 10-14 days.

The experiment is repeated at least 3 times. Test-mycobacteria should be are resistant to heating at 60 ° C for at least 1 hour.

Determination of resistance of test-mycobacteria to reference DS

To determine the resistance of mycobacteria as a The reference DSs use monochloramine B, glutaraldehyde and hydrogen peroxide medical.

The iodometric titration method is used to determine the percentage active chlorine in monochloramine B. In the experiments, containing 26.0-28.0% active chlorine, dissolving which in distilled water in a ratio of 1:20 prepare a working solution (5.0% by weight).

Glutaraldehyde (registered in Russia as Substance) is diluted with distilled water to a concentration of 0.5% working solution (glutaraldehyde).

The mass fraction of hydrogen peroxide is determined by the medical method of permanganatometric titration in accordance with with GOST 177-88. Prepare a 4.0% solution (for hydrogen peroxide).

Prepare and pour into tubes of 5 ml sterile neutralizer solution (clause 2.1.1.4.); sterile drinking water; test tubes with beveled dense nutrient medium Levenshtein-Jensen or "New". Prepare and contaminate the test-mycobacteria, bath test test objects (see 1.2.2.2.). If in

use previously prepared and stored in
refrigerated test-microbial test-objects, then their

are removed from the refrigerator in advance so that they can purchase a room
temperature (18-20 ° C).

Prior to setting up the experiment, the content of LW in
working solution DS.

When carrying out experiments in a glass container with a volume
50-100 ml with a pipette pour the required volume of a solution of the reference
DS, at a rate of 0.5 ml for each test object and placed in a water
Bath with a temperature of 20 ° C for the entire period of the experiment. Count in a cup
Petri needed for experiment amount of coarse (cambric)
test objects (2 for each exposure), contaminated test-
Mycobacterium, captured with sterile tweezers test objects all
immediately and lower them into a container with a solution of DS; light wiggle
capacities achieve full wetting of test objects. In the moment
wetting of all test objects mark time.

Every 30 minutes. Sterile tweezers extract 2 test-
of the object from the DS solution and drop them into a test tube with 5 ml of 1.0%
sterile solution of sodium thiosulfate for neutralization
the residual effect of DS. A test tube with a neutralizer and no tests
is shaken. After 5-10 minutes. test objects are transferred to
test tube with 5 ml of sterile drinking water. After another 10-15 minutes.
each test object is placed on a surface slanting in a test tube
dense nutrient medium.

To control two contaminated test-mycobacteria test-
of the object is immersed in sterile drinking water (instead of a solution of DS)
for a maximum exposure period, then (as well as experienced test objects)
they are transferred to a neutralizer solution (sodium thiosulfate) and
are placed on the surface of a dense,
environment.

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Experimental and control sowings are placed in a thermostat at temperature 37 ° C; The presence of growth of test cultures is checked in 14-21 days.

The experiment is repeated at least 3 times. Test-mycobacteria should be resistant to a 5.0% solution of monochloramine for at least 2 hours; to 0.5% solution glutaraldehyde - at least 60 minutes; to a 4.0% solution hydrogen peroxide - not less than 60 min.

In the process of testing, the temperature of the solution of DS in experience, the initial and residual levels of contamination test-mycobacteria of the test object (CFU / cm^2), the test-objects in the tested disinfectant solution.

1.2.1.4 Ensuring the standard conditions for conducting research activity of DS and their substances

For ensuring the standard conditions staging experiments it is necessary to carry out chemical-analytical control investigated DS and their substances. Before the study tuberculocidal activity of DS and their substances is necessary read the formulation of the product and the technical specifications for domestic or a specification for foreign funds, chemical-analytical studies to determine the concentration substances in the media and determine compliance recipes and other indicators, regulated the above documents. In doing so, the chemical-analytical methods of control and apply storage conditions means and security measures when working with it, proposed the manufacturer of the facility.

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Since for the disinfection of tuberculosis, possessing action, killing mycobacteria, a not delaying their growth, in determining tuberculocidal activity

and the effectiveness of the DS should distinguish tuberculocidal action of the drug against tuberculostatic. To do this, apply neutralizers that exclude residual bactericidal action.

To neutralize the antimicrobial action of disinfectants funds from different chemical groups apply the following neutralizers:

for haloactive (chloro-, bromo- and iodoactive) and oxygen (hydrogen peroxide, its complexes with salts, peroxyacetic acid, ozone) 0.1-1.0% solutions of sodium thiosulfate;

for Quaternary ammonium salts (alkyldimethylbenzylammonium chloride, didecyldimethylammonium chloride and etc.), derivatives guanidine (polyhexamethyleneguanidine hydrochloride, chlorhexidine bigluconate, etc.) - 0,1-1,0% solutions sodium lauryl sulfate (sulfonol), solutions of sodium lauryl sulfate with 10% skimmed milk or a universal neutralizer. below);

for aldehydes (glutaraldehyde, glyoxal, formaldehyde, orthophthalic aldehyde) - 1.0% solution pyrosulphite (metabisulphite) sodium or a universal neutralizer (see below);

for acids - alkali in an equivalent amount;

for alkalis - acids in an equivalent amount;

for alcohols - dilution in water to non-active concentration;

for composite agents - universal neutralizer, containing Tween 80 (3%), saponin (0.3-3%), histidine (0.1%), cysteine

(0.1%). If the composition contains oxidants, the neutralizer additionally, sodium thiosulfate is added. Universal The neutralizer is also a neutralizing broth according to Di-Inli (company-manufacturer "HIMEDIA"). It includes such ingredients like casein hydrolyzate, yeast extract, glucose, sodium thiosulfate, sodium thioglycolate, sodium bisulfite, lecithin, Tween 80 and others.

Neutralizer solutions are sterilized by autoclaving at 1.1 atm. (121 ° C) for 15 minutes.

The temperature of neutralizer solutions should be 20 ° C,
regardless of the ambient temperature.

Ready-to-use solutions should be used on the day of preparation.
Storage of ready solutions at a temperature of 4 ° C
within 48 hours.

Control of completeness of neutralization of residual action
tested DS.

Despite existing recommendations for use
neutralizers for various active substances (DV), many
Modern DSs contain several DVs and other auxiliary
substances, possessing bacteriostatic action, and
the existing (recommended) neutralizers may not
to effectively neutralize the residual effect of the DS in
sample. In this regard, the results of evaluating the effectiveness of the DS can be
biased.

Therefore, every case of a DS test should
preliminary accompanied by experimental control
effectiveness of neutralization of residual effect of DS on microbial
cell.

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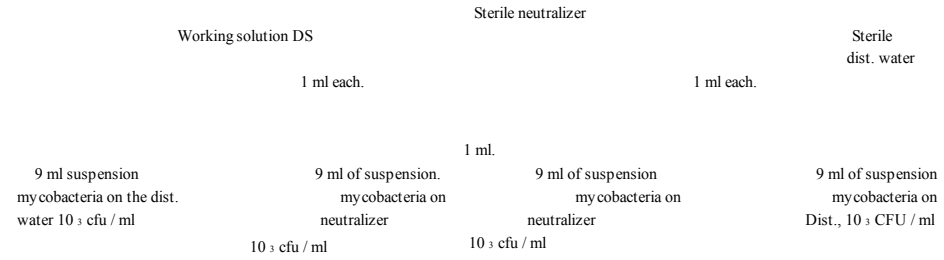
To monitor the effectiveness of the neutralizer and completeness
neutralization DS use suspension method,
which provides for the study, basic operations
which and their purpose are shown in Figure 1.3 and in Table 1.6.

Diagram 1.3.

Conducting an experiment to control the effectiveness of neutralization
effects of DS on mycobacteria used by the neutralizer



1 2 3 4

Test tubes with Levenshtein-Jensen cut or dense nutrient medium or "New"

Table 1.6.

Assignment of an experiment to evaluate the effectiveness of neutralizing the residual effect of DS

No. samples	Purpose of the operation research	Execution procedure research operations	Expected result
1.	Control of the destructive DS actions	to 9 ml of a suspension of test-strain (10 3 cfu / ml) at dist. water + 1 ml solution DS	Growth microorganisms must absent
2.	Control of completeness neutralizing the DS	to 9 ml of a suspension of test-strain (10 3 cfu / ml) at neutralizer + 1 ml solution of disinfectant	About the same quantity colonies in crops

3.	Control of absence antimicrobial effect of neutralizer	to 9 ml of a suspension of test-strain (10 3 cfu / ml) at neutralizer + 1 ml neutralizer solution	samples (0.1 ml each) at dense nutrient medium
4.	Reference Control quantities mycobacteria	to 9 ml of a suspension of test-strain (10 3 cfu / ml) at dist. water + 1 ml dist. water	

Note: 5 minutes after the experiment, from each of the four samples produce a mixture of 0.1 ml, at least 3 tubes with beveled a nutrient medium that is incubated in a thermostat at 37 ◦ C, after 14-21 days take into account the results of research.

1.2.2 Methods for researching and evaluating the results of DS activity and their substances in vitro

1.2.2.1. Suspension method

Suspension method for evaluation of tuberculocidal activity of DS and their substances are used to obtain primary information about concentration and time parameters of effective viable mycobacteria) tuberculocidal effect of DS. Methodology for the implementation of the tuberculocidal activity of the DS by the suspension method is shown in the scheme figure 1.4.

As can be seen from Scheme 1.4, in order to carry out the evaluation experience

tuberculocidal properties of the disinfectant suspension
method must be prepared:

a working suspension of a test-mycobacterial strain with concentration not less than 1 · 109 cfu / ml;

Test tubes with sterile drinking water for monitoring the actual biological concentration (BK) of the test microorganism in suspension used in the experiment;

test tube or vial with a solution of DS in the test concentration in the amount necessary to ensure the selection of all samples;

required number of tubes (depending on the amount samples taken to determine the time death of the test microbe), containing 9 ml of neutralizer, previously tested for neutralization efficiency residual effect of the DS under test (clause 1.1.2.3.);

test tubes with a chopped sterile dense nutrient medium in quantity required for seeding the control sample of the original suspension and control samples of the effectiveness of the DS action on the test microbe.

Scheme 1.4.

Conducting an experiment to assess the tuberculocidal activity of DS suspension method

Preparation suspensions test-mycobacteria and control biological their concentration in it by consecutive 10-fold dilution and sowing on dense growth medium

Preparation of working solution DS Mixing suspensions test-mycobacteria and disinfectant solution in a ratio of 1: 9. exposition impacts DS on mycobacteria .

Sampling from a mixture of 3 samples (1 ml each) after each exposure and transfer them to test tubes from 9 ml neutralizer residual effect of DS

Shaking samples on the shaker. Sowing from each sample by 0.1 ml per dish with nutrient medium Thermostating crops

Test tubes with sterile to drink alcohol

A test tube with a working suspension test microbe in concentration (	water (9 ml each) in quantity, necessary for determine the BC test-microbe in suspension	Petri dishes with dense nutritious medium (5 cups for the sample)	A test tube or vial with working solution of DS	Test tubes with 9 ml neutralizer (not less than 3 per exposure)	Petri dishes with dense nutritious medium (not less than 3 per each sample)

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The methodology for carrying out the experiment itself includes, as can be seen from schemes, sequential execution of the following operations:

careful mixing of stored in vitro or in vial working suspension of the test microorganism by shaking it in 2-3 minutes;

putting the test solution of DS in the water bath with a given temperature; if the task of the experiment is not provided The study of the effect of temperature on the efficacy of the agent, then the evaluation of the effectiveness of the test solution solution is carried out at a temperature of 18-20 ° C;

control of the real at the time of the experiment biological concentration (BC) of the test microorganism in suspension;

insertion into the tested disinfectant solution suspension of the test microorganism with the ratio of DS and suspension of the test microorganism 9: 1;

Mixing of the mixture and counting by stopwatch start time exposure of the DS to the test microorganism;

at the end of each given exposure, selection sample in the amount of 3 ml, which 1 ml is added to 3 tubes, containing 9 ml sterile residual solution neutralizer the action of the disinfectant on the test microorganism;

stirring the sample by shaking by hand for 1-2 min. (or within 5 minutes on the shaker) and seeding from them sterilely on the surface of a dense nutrient medium in Petri dishes or in test tubes (0.1 ml per cup or tube, but not less than on three of each sample).

incubation of the culture of the samples at a temperature of 37 ± 1 ° C for 14-21 days and recording results.

Effective exposure for working solution tested concentration is considered the second exposure of the lack of viable cells in the crops of the corresponding samples.

The agent, solutions of which are provided at room temperature temperature for 60 min. complete death of mycobacteria, are considered as a promising tuberculocidal agent for further studying: assessments factors, influencing on tuberculocidal activity of DS, development of effective applications, etc.

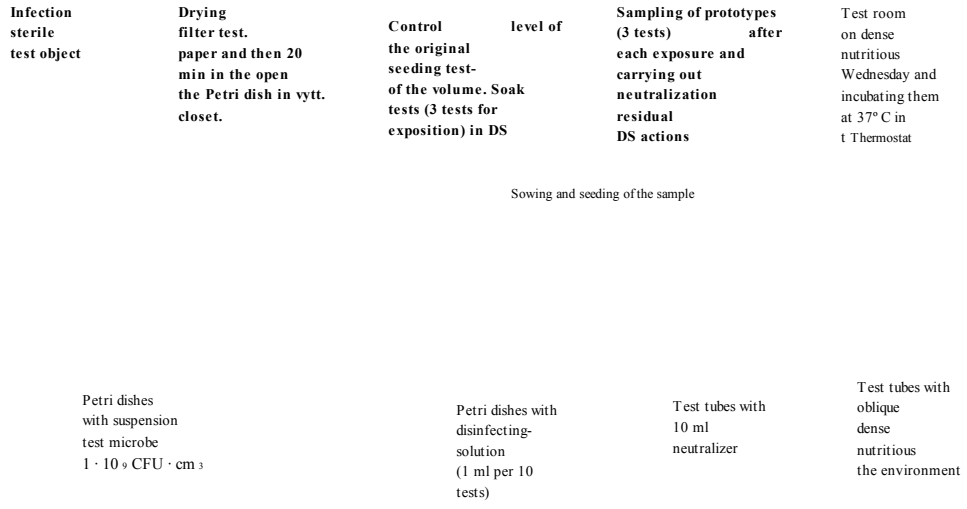
1.2.2.2. The method of cambric test objects

As a suspension method for evaluating tuberculocidal activity DS and their substances, and the method of the batistovye test objects use for information on concentration and time parameters of effective tuberculocidal action of DS. AT in principle, the methodology for carrying out the evaluation (testing) of tuberculocidal efficacy of DS by sampler test objects is shown in figure 1.5.

As can be seen from the scheme 1.5., The experimental procedure provides carrying out of contamination by mycobacteria of batist test-objects, control the initial level of seeding of test objects, processing (soak) test objects in the tested disinfectant solution, neutralization of DS after a given exposure exposure, Incubating neutralized test objects on a dense nutrient medium.

Scheme 1.5.

Performance of the experiment on evaluation of tuberculosis activity disinfectant by the method of cambric test objects



Preparation and contamination of cambric test objects.

Batist tissue is immersed for 24 hours in a cold drinking water to remove the coupling agent. Then the fabric is carefully washed with soap, rinsed in cold water, boiled, dried and patted hot an iron. Using a needle in a prepared piece of cambric tissue pull the threads in the longitudinal direction at a distance of 10 mm from each other and in the transverse - at a distance of 0.5 cm. On these lines The fabric is cut with scissors into separate test objects; lay out 50 pieces in Petri dishes, which are wrapped in paper and autoclaved in a steam sterilizer for 20 minutes. at 132 ° C (1.1 kgf / cm 2).

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Prepare working slurry test-mycobacteria (clause 2.1.1.2.) in an amount sufficient to contaminate the used in the test-objects test (at the rate of 0.2 ml per test).

Sterile batistovye test objects (in an amount of 50-100 pieces) in a Petri dish, 10-20 ml of a working suspension of test-mycobacterium, containing 10 9 CFU / ml, and evenly wetting, The test objects are left in suspension in a closed Petri dish. Across 30 min. test-objects, contaminated with bacterial suspension,

Sterile pincers with aseptic compliance are transferred to a sterile a petri dish on the surface of a two-layer sterile filter paper, cover them with a sterile filter paper and close the cup. Leave for 10 minutes. in order to remove excess liquid. To fix microorganisms on the batist test-objects, the latter is transferred to the surface of a dry sterile filter paper in a Petri dish and cover from above with a sterile sheet of filter paper, dried in a thermostat at 37 ± 1 ° C for 20 min. with a slightly open lid.

Storage of contaminated test objects is possible in The refrigerator at a temperature of 4 ± 2 ° C during the day.

Control of the initial number of living test-mycobacteria on the test object

To control the number of viable mycobacterial cells on test objects, the test object is immersed in a flask with 100 ml sterile drinking water. The flask is placed on a shaker and shake for 30 minutes. for the washing of bacterial cells with bazevogo (batistovogo) test object. From the obtained suspension produce 0.1 ml culture for 5 tubes (5 replicates) with medium Levenshtein-Jensen or Nova, are incubated in a thermostat at 37 ° C

within 14-21 days. Growing colonies on the surface of nutrient The medium is counted, the mean value is determined, the recounting taking into account breeding. Number of viable bacterial cells in the prepared suspension at the test site should be 10^5 -10^6 microbial bodies (if the growth of mycobacteria It is absent, when sowing a 0.1 ml slurry, it can be increased volume to 0.2-0.3 ml and, accordingly, take it into account in the calculation bacterial concentration on the test facility).

Calculation of the concentration of live mycobacteria at the test site is carried out according to the following formula:

X = A x 1000, where

X - concentration of live mycobacteria at the test site;

A is the average number of colony forming units (CFU),

grown on 5 test tubes;

1000 - the coefficient obtained from the ratio of 100 ml (total volume of sample in a flask) to 0.1 ml (sample volume used for sowing).

For example:

the growth of mycobacteria in the first sample was 50 cfu, in the second - 110 CFU, in the third - 98 CFU, in the fourth - 150 CFU, in the fifth - 100 CFU, then the average number of constituent units (CFU), grown on 5 test tubes will be:

A = (50 + 110 + 98 + 150 + 100): 5 = 102

X = 102 × 1000 = 10,200,000, which corresponds to 1 million live microbial cells on the test object.

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The processing of test objects by the solution of the test DS and control of decontamination efficiency

Sterile tweezers immerse the necessary for carrying experiment quantity contaminated test strain mycobacterium test objects in a test tube or a petri dish with a solution DS (the volume of DS should be calculated taking into account the ratio of 1.0 ml solution for 1 test object), providing complete wetting (immersion) of test objects, record the time of the beginning of the exposure and follow the observance of the temperature regime (20 ± 2 ° C).

After the set exposure time, sterile Tweezers extract the test object from the solution of DS and immerse it for 1-2 minutes. into a test tube with a sterile neutralizer solution under temperature of 20 ± 2 ° C (a test tube with neutralizer solution and immersed in a test do not shake).

Then, using a pair of tweezers, place the test object on the beveled the surface of the Levenstein-Jensen nutrient medium or the "New" (on the bevelled area of the culture medium in a test tube, place 3 test objects, which can be increased without

additional costs, the number of samples for each exposure, and
means, and reliability of results of an estimation of efficiency of means).

The tubes are covered with cotton-gauze stoppers, placed in
Thermostat in an inclined position at an angle of 30º so that
Drainage from the dough was equally distributed
on the surface of the culture medium, and incubated at 37 ± 1 ° C in
for 10-21 days with daily viewing.

Accounting and analysis of experimental results

The calculation of the results of crops is carried out visually by counting
grown colonies on the test site itself and on the surface

nutrient medium. Colonies of M. terrae on Levenstein-Jensen medium
are rough and matte convex rounded
education, on the "New" environment - smooth and shiny or with milk
or a yellowish tinge.

The presence of growth of colonies of test-mycobacteria at the test facility and
surface of the culture medium shows that the test DS in
In this application mode (solution concentration and exposure
impact) does not provide a reliable tuberculocidal effect.

Absence of growth of colonies of test-mycobacteria at the test facility and at
The surface of the nutrient medium indicates the presence of
This mode (concentration of solution and exposure exposure)
tuberculocidal effectiveness, responding the
requirements for DS for practical use (provision of
decrease in the level of seeding of the object by 10^5 CFU · cm^2).

Effective exposure for working solution tested
concentration is considered the second exposure of those who showed no
sowing the corresponding samples of viable mycobacteria.

The number and interval (step) of exposures at which
sampling is carried out for the effectiveness of the DS,
data on the composition and effectiveness of the
substances.

The agent, solutions of which are provided at temperature
20 ± 2 ° C for 60 min. complete death of mycobacteria test-
microorganism, can be considered as promising

tuberculocidal DC for further study:
influencing tuberculocidal activity of DS, regimens
effective application, etc.

1.2.2.3 Methods for studying factors affecting
tuberculocidal activity of DS and their substances

To determine the application conditions and directions for further
It is necessary to study the dependence of tuberculocidal
the effect of DS on the temperature of the DS solution, the pH value and the presence
protein contamination.

Studies are carried out using the method of bathist test objects
(clause 1.2.2.2.).

Determination of the effect of temperature on tuberculocidal
activity of DS and their substances is carried out with the purpose of revealing
possibilities of using heated solutions of DS to reduce
time of disinfection of objects against mycobacteria
tuberculosis, as well as to assess the effectiveness of tuberculocidal
properties at low ambient temperatures,
disinfected object and the DS solution itself.

To study the effect of temperature, working solutions
subjects DS are prepared on the day of the experiment, poured into glass flasks
(test tubes) at a rate of 0.5 ml for each test object.

Investigation of the influence of positive temperatures of the DS solution on
its tuberculocidal activity is carried out using a water
bath, in which a container with a disinfectant solution is heated
up to 18 ± 1 ° C, 37 ± 1 ° C, 55 ± 1 ° C, after which the infected
test objects and maintain these temperatures throughout the experience.

Experiments to assess the effect of reduced temperature on
the activity of the DS is carried out using a cryostat or saline
low-freezing solutions in which the container is cooled with
disinfectant solution up to 10 ± 1 ° C, 5 ± 1 ° C, minus 2 ± 1 ° C and
keeping them in the process of experience. After reaching the

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temperature in a solution of DS submerged batistovye test objects, contaminated culture of the test microorganism from the calculation of 2 test-object for each exposure.

At regular intervals from each flask 2 test-objects are taken out and placed at a temperature of 20 ± 1 ° C in tubes with an appropriate neutralizer for 5 minutes, then in a second test tube with sterile water for 5 minutes. and only after this Each test object is transferred to a test tube with a beveled dense Levenshtein-Jensen nutrient medium or "Novaya" and stack its on the surface of the medium. The crops are incubated for 14-21 days at 37 ± 1 ° C. Control serves by 2 test objects at each investigated temperature, not subjected to the action of the subject DS, but immersed in test tubes with sterile drinking water for a period of, equal to the action of the subject DS.

Determination of the effect of pH on tuberculocidal the activity of DS and their substances begins with the preparation of workers solutions of DS with different pH values. For acidification solution using a decinormal solution of hydrochloric or other acid, and for alkalization - decinormal alkali solution. AT prepared by solutions immersed contaminated Mycobacteria are bathist test objects. The study of dependence tuberculocidal activity of DS and their substances from the pH value are carried out according to the procedure described above, only when neutralizing actions of active substances simultaneously reduce or increase and the pH value, adding respectively the acid or alkali.

Determination of the effect of protein contamination on tuberculocidal activity DS is conducted in order to identify the possibility of influence (or

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of establishing him absence) protein pollution on disinfected facility for tuberculocidal activity of the DS.

The study is carried out using the method of cambric test objects (clause 1.2.2.2.), only for the contamination of test objects are used a suspension of test-mycobacteria containing 20.0% of inactivated serum of cattle or defibrinated blood, which are added to the slurry during its preparation. Inactivation normal serum of bovine animals is carried out in fractional three times warming in a water bath at a temperature of 60 ± 1 ° C in within 30 minutes. If the activity of the drug does not decrease in the presence of 20.0% protein, the concentration of inactivated large serum cattle or defibrinated blood in a suspension of test- the microorganism is increased to 40.0%. No reduction tuberculocidal activity of DS with addition of 40.0% serum allows to consider DS not reacting to the presence of protein pollution.

1.2.3 Methods of studies of tuberculocidal efficacy of DS, intended for disinfection of objects of the external environment, contaminated with test microorganisms

List of test objects modeling objects subject to disinfection, includes: surfaces of premises, furniture, apparatus, devices, sanitary equipment, transport means, etc .; medical products, including endoscopes; subjects of nursing, toys; utensils, including laboratory and from under the discharge; Linen, clothes, overalls and other objects from the tissues; rubber products, including gloves, boots, aprons, etc .; footwear; Hands in rubber gloves; remnants of food;

allocation: feces, urine, blood, sputum; water; air; medical waste.

1.2.3.1 Study of tuberculocidal efficacy of DS, intended for disinfection of surfaces of premises, furniture, apparatus, instruments, sanitary equipment, vehicles and other objects

In the studies, test surfaces (10 × 10 cm) from

various materials: wood (unpainted, painted with oil,
glue or other paints, covered with wallpaper), linoleum,
plastic, tiles, faience, tiles, metals, glass.

Test-surfaces of various materials (except for
wooden test surfaces, painted with glue and
covered with wallpaper) thoroughly washed with water and soap and a brush,
sterilize in a steam sterilizer (at a temperature of 121 ± 1 ° C in
for 30 minutes). Test surfaces, painted with glue and
wallpapered, wallpapered several times with a sterile gauze
a napkin moistened with sterile drinking water. Prepare
a suspension of test-mycobacteria containing $2.0 \cdot 10^9$ cfu / ml. When
development of disinfection regimes for shells, bathtubs and other
contaminated sites to simulate organic pollution
40.0% horse serum, inactivated
at 56 ° C for 30 minutes. (to 6 ml of a 2-billion suspension
add 4 ml of serum).

After drying, the test surface is positioned horizontally and
on them using a single-channel mechanical dispenser or
0.5 ml of the suspension of the test microorganism is applied to the glass pipette.
The suspension is uniformly distributed over the test surface (100 cm^2)
sterile glass spatula. If suspension test-

the microorganism is not evenly distributed, but is collected in a drop,
rubbing by a spatula on the test surface is carried out repeatedly
(3-5 times). Contaminated test surfaces are dried
room temperature until completely dry (30-120 minutes).
Disinfection test surfaces implement ways
wiping, irrigation with disinfectant solution.

When disinfecting the test surface from wood (painted
glue and other paints, pasted wallpaper), glass, tiles and
others are placed vertically; surface from linoleum, metlakh
tiles and other floor coverings are placed horizontally. Test-
surfaces are disinfected by irrigation, wiping
(single or double) disinfectant solution.

Depending on the type of surface to be treated and availability contamination on it, the rate of DS consumption per treatment by wipe is 100-150 ml per m^2 (1-1.5 ml per 100 cm^2); way irrigation - 150 ml per m^2 (1.5 ml per 100 cm^2) during processing with a "Kvazar" sprayer and 300 ml per m^2 (3 ml per 100 cm^2) - when spraying of the "AutoMax" type or a water jet.

If necessary, rubbing or Irrigation is repeated after 5-15 minutes.

For control effectiveness of disinfection across certain time intervals (15-30-60, etc. to 120 min.) with the test-Surface washings are done by careful wiping surface with a sterile gauze cloth (5 cm^2), moistened neutralizer. After wiping on the test surface should not remain excess moisture. Napkins are immersed for 5 minutes. in test tubes (capacity) with an appropriate neutralizer (10 ml), and then in Sterile drinking water with beads and shaken on a shaker

within 10 minutes. The resulting flushing liquid is introduced into 0.1 ml in 3-5 tubes with mown dense nutrient medium Levenstein-Jensen or "New", carefully distributing it throughout the surface. The cultures are incubated in a thermostat at a temperature of 37 ± 1 ° C within 10-21 days.

AT control experiments for processing similarly contaminated test surfaces instead of the solution of DS are used Sterile drinking water from the same calculation as the experimental ones. A liquid into which a sterile gauze pad is placed after take the flush from the control surfaces, before planting, dilute in 100 times and add 0.1 ml to the sloping surface of a dense Levenstein-Jensen medium or "New" 3-5 test tubes. The cultures are incubated in a thermostat at a temperature of 37 ± 1 ° C. Take into account results after 10-21 days.

Evaluation of the results is carried out on the sowing of that breeding, in the number of colonies on a Petri dish or in a test tube is from 30 to 300.

After standing in the thermostat, the number

colonies on plates or test tubes with a dense nutrient medium, the residual density of contamination per 100 cm 2 of test-surface and calculate the effectiveness of decontamination, taking the number of test microorganisms taken from the control test-objects (test-surfaces), for 100%.

For example:

per 100 cm 2 control test surface by results 148,000 microbial cells were detected in bacteriological control, and on a similar type of experimental test surface-20 microbial cells.

148,000 - 100% x = 20x100: 148,000 = 2: 148 = 0.013%

20s

The effectiveness of disinfection of the test test surface is:

100 - 0.013 = 99.987%.

Criterion for the effectiveness of DS in disinfecting test-surfaces from various materials, contaminated test-microorganism is not less than 99.99%.

1.2.3.2. The study of tuberculocidal efficacy of DS, intended for disinfection of objects of care for patients and toys of various materials (except soft)

In studies, test objects (100 cm 2) and objects care for patients from various materials: rubbers based on natural and silicone rubber (medical oilcloth, hot-water bottle, pear); glass (drinker, spittoon, thermometer); plastics (heating pad, tray, tip for enemas); metals (a basin, a glass for thermometer); toys (except soft) from rubber and plastics.

Test objects, nursing and toys from various materials are thoroughly washed with water and soap and a brush. Test-Objects are sterilized by steam or air.

Prepare slurry test-mycobacteria, containing $2.0 \cdot 10^9$ CFU $\cdot$ ml $^{-1}$. To the suspension was added 40.0% horse serum, inactivated at 56 ° C for 30 minutes (to 6 ml of 2-billionth

of the suspension are added 4 ml of serum).

Using a single-channel mechanical dispenser or
A glass pipette was applied to the surface of the test object with 0.5 ml
suspension of the test microorganism. The suspension is evenly distributed

on the surface (100 cm^2) with a sterile glass spatula. Channels and
cavity of the subject of nursing, toys are filled with
syringe. Small toys are completely immersed in the suspension.
Contaminated test objects, nursing and
the toys are dried at room temperature until complete
drying (30-120 minutes).

DS solutions are prepared at tap water at a temperature of 20 ± 2 ° C.
Disinfection is carried out by a method of immersion, rubbing,
irrigation. After drying, the contaminated test objects,
goods for care of patients, toys, including those with channels and
cavity, immersed in a solution of the tested disinfectant
means or wipe with a tissue moistened with it. Small Toys
completely immersed in a container with a solution of DS, preventing them
surfacing; large toys are disinfected by irrigation.
The rate of DS consumption by wiping method at the rate of 100-150 ml / m^2 at
single treatment and 200-300 ml / m^2 for a double; way
irrigation - 150 ml / m^2 when treated with a "Kvazar" type atomizer, with
spraying of the "AutoMax" type or a water trap -
300 ml / m^2 . If necessary, rubbing or
Irrigation is repeated after 5-15 minutes.

For control effectiveness of disinfection across
certain time intervals after treatment (30-60-120 minutes).
Test objects, nursing and toys are extracted from
solution, make washings with a sterile gauze cloth (5 cm^2),
moistened with a neutralizer. The napkins are immersed in a sterile
neutralizer solution for 5 minutes, then transferred to test tubes
(capacity) with beads and sterile drinking water (10 ml) and shake
on the shaker for 10 min. Channels and cavities are washed

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neutralizer (10 ml), which is collected in sterile tubes
(capacity) and left for 5 minutes. for neutralization.

The resulting flushing liquid and flush fluid from the channels
add 0.1 ml to the chamfered surface of a dense nutritious
Levenstein-Jensen medium or "New" 3-5 test tubes. In the control
Instead of a solution of DS, sterile drinking water is used.

The cultures are incubated in a thermostat at a temperature of 37 ± 1 ° C.

The results are taken into account after 10-21 days of incubation.

The effectiveness of DS in disinfecting test objects,
subjects of care for patients and toys from various materials
(other than soft) contaminated with the test microorganism should
be at least 100%.

1.2.3.3 Study of tuberculocidal efficacy of DS,
intended for disinfection of tableware, laboratory
and from under the secretions

For definitions tuberculocidal activity DS,
intended for the disinfection of dishes, is used as a
test objects a set of a dining room and tea utensils: plates, glasses,
mugs made of various materials (porcelain, faience, aluminum, glass,
plastic, dishes covered with enamel); cutlery: knives, forks,
Spoons from a variety of materials (stainless steel, aluminum,
plastic), disposable dishes and a set of laboratory
utensils, representing test objects of glass and plastics:
Subject and cover glasses, pipettes, Petri dishes, plates for
immunological analysis and other; crockery
(urinals, bedpan). Before experiment, utensils and
Cutlery is washed with water and soap and a brush and dried.

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On the dishes (an area of 100 cm ^{2}), a test-

microorganism at the rate of 0.5 ml of a suspension containing $2 \cdot 10^9$ cfu in 1 ml. The suspension of the test microorganism is uniformly distributed over the dish surfaces with a glass spatula. Cutlery for the contamination is immersed in a bacterial suspension for 1-2 minutes, leaving their pens unblocked. Contaminated dishes dried (until completely dry) at room temperature (30-120 minutes) and relative air humidity of 50-60%.

For the development of disinfection regimes for dishes with residues food during contamination use a suspension of the test microorganism, mixed with oatmeal, semolina or other porridge, boiled in milk with butter (10 g of porridge is added 1 ml of 2-billionth microbial suspension). To simulate the contamination of tea utensils use jelly (10 g of kissel add 1 ml of 2-billion microbial suspension), laboratory dishes - 40.0% inactivated serum, utensils from the discharge - 20.0% emulsion of feces, previously rubbed in a mortar.

Processing of a dining room, tea, laboratory utensils, dining rooms devices are carried out by immersion in a disinfectant solution. The solutions are prepared in drinking water. The temperature of the test solution 18-20 ° C. If necessary, study the effectiveness of solutions of DS at temperature of 50 ° C.

The disinfectant solution must be completely filled with excessively cover all dishes and appliances (at a rate of at least 2 liters for 1 set).

The disinfection time of the dishes is from 15 to 240 minutes, depending from the type of DS and the presence of contamination.

After certain time intervals (for example, 15, 30, 60 min. , etc.) extract one item of different names (for example, a plate, a glass, a slide, a knife, etc.) from a disinfectant solution and a sterile gauze (5 cm^2) soaked in solution of the neutralizer corresponding to this DS, carefully wipe the infected part of each object and immerse in 10 ml

of the same neutralizer for 5 minutes, then the napkin is transferred to a test tube with sterile drinking water and beads. Waste time gauze napkins - 10 minutes. with constant shaking on the shaker. After washing, a wash liquid of 0.1 ml is sown in 3-5 test tubes with a sloping surface of a dense nutrient medium of Levenshtein-Jensen or "New" (0.1 ml each). Crops are placed in thermostat at a temperature of 37 ± 1 ° C. Preliminary accounting of results spend 10-14 days, the final - after 21 days.

Control is similarly contaminated dishes, which is not immersed in a disinfectant solution, but in the same volume of sterile drinking water.

Criterion for the effectiveness of disinfection of dishes: the death of test-microorganism is not less than 100%.

1.2.3.4 Study of tuberculocidal efficacy of DS, intended for the disinfection of medical devices (MI), including endoscopes

1.2.3.4.1. The study of tuberculocidal efficacy of DS, intended for the disinfection of medical devices (except for endoscopes)

As test products use sterile tools and Other MIs, including single use, from various materials (metals, rubbers, glass, plastics) or imitating them

test objects. The list of tools should include a variety of by the shape, nature of the surface and the material of the product used (smooth products of a simple configuration, products with lock parts, channels and cavities with incisions and sputtering, products convoluted shape; products made of several types materials, etc.).

On the working surface of the test product (for lock products - also in the area of the castle, and in the presence of channels and cavities - also in the channel of the article), 0.1 ml of a suspension containing 2×10^9 cfu / ml test microorganism containing 40% inactivated horse serum. Small test products for contamination are immersed in this suspension for 15 minutes. Contaminated test products are dried in

thermostat for 20-25 minutes. Disinfectant solutions are prepared on drinking water at room temperature or heated to 50 ± 1 ° C.

After drying, the contaminated products are immersed in solution of the test substance, filling it with all channels and cavities products, avoiding the formation of air congestion. Instruments, having lock parts, immerse open, previously making them in the DS solution a few working motions for a better penetration of the solution into hard-to-reach areas of the product in the area castle. The thickness of the solution layer above the product should not be less than 1 cm. Contaminated control products in parallel immersed in water.

After a certain time (from 15 to 120 minutes), the products are removed from the solution and a gauze pad measuring 5 × 5 cm, impregnated neutralizer, the surface of the product make rinses, napkins is placed in a test tube with 10 ml of the same neutralizer for 5 minutes, then Transfer it to a test tube with sterile drinking water and shake it off.

beads for 5-10 minutes. To monitor the effectiveness of Disinfection is done by flushing a 0.1 ml the surface of the agar nutrient medium, and the napkin is placed in bouillon. The product channel is washed with a neutralizer, and the flushing the liquid is seeded in 0.1 ml onto the chopped dense nutritious medium in 3-5 test tubes. The crops are kept in a thermostat at temperature 37 ± 1 ° C for 21 days. Accounting for preliminary the results are carried out after 10-14 days and the final results - after 21 day.

The multiplicity of the statement of the experiment must be sufficient for obtaining statistically reliable results.

The regime (concentration-time-temperature) is considered to be effective; providing 100% death of test microorganisms on all products.
In the presence of positive samples, the experiment is repeated, increasing concentration or time of exposure.

1.2.3.4.2. The study of tuberculocidal efficacy of DS,

intended for disinfection of endoscopes

As test objects, fragments of the endoscope or endoscope (flexible - gastroscope, hard - cystoscope). To 0.1 ml suspension containing $1 \cdot 10^9$ cfu / ml of test microorganisms is applied with using a pipette on the outer surface and into the endoscope channel, dried for 20 minutes. Then the contaminated product immersed in a solution of DS, filling the cavities and channels of the endoscope. Across A certain time (15 to 60 minutes) the product is removed from the solution and wash the outer surface with a gauze cloth (5 × 5 cm), wetted in neutralizer solution; the napkin is placed in a test tube with 10 ml of the same sterile neutralizer solution for 5 minutes, and then

transfer it into a test tube with sterile drinking water, and shake it with beads for 5-10 minutes. The product channel is washed with a solution The neutralizer and flush fluid are seeded on nutrient agar.

Control of microbial contamination of used for washing the samples of neutralizer solution and drinking water. Multiplicity The experiment should be sufficient to obtain statistically reliable results.

The regime (concentration-time-temperature) is considered to be effective; ensuring the death of the test microorganism on all test products and absence of test microorganisms in the neutralizer solution. When The presence of positive samples of the experiment is repeated, increasing exposure time, but no more than up to 60 min.

Positive is a sample showing a characteristic growth test microorganism on dense nutrient media or detection test microorganism in the solution of the neutralizer used.

The disinfection regime, developed on channel simulators endoscope, check at disinfection endoscope, contaminated test microorganism. If necessary effectiveness of the developed regime is tested in practical conditions.

Criterion for the effectiveness of DS (mode of application of DS) for disinfection of medical devices (including endoscopes) is 100% death

test-microorganism.

Research tuberculocidal efficacy DS in disinfection

high level endoscopes

This methodology is designed to develop modes
disinfection of a high level of endoscopes during
"Non-sterile" diagnostic and therapeutic manipulations.

For the study, a DS possessing a sporicidal
action, and it is tested under the same conditions
(concentration, temperature), which ensure the death of the spores
bacilli, determining the necessary disinfection time for
the attainment of the death of the most stable to the studied DS
microorganism.

In determining the effectiveness of the means for endoscope TLDs in
As the test objects, fragments of the flexible channel
endoscope or its imitators in the form of tubes of plastic 2 cm in length,
with a diameter of 2 mm.

Sterile test objects are artificially contaminated,
Using the dispenser / micropipette to the central part of the channel and
the surface of each tube is a suspension of the test microorganism from the calculation:
106 microbial cells of test-mycobacteria for each product. For
imitation of organic contamination to a suspension of microorganisms
before contamination of the object add 2% inactivated
horse serum.

The contaminated test objects are dried at room temperature
temperature 18-22 ° C for 20 minutes.

The disinfectant solution is prepared on a sterile tap
(drinking) water. Processing of the DS under investigation is carried out by the method
immersion in the test disinfectant solution. Across
certain time intervals, depending on the chemical composition
means, in the interval from 5 to 60 minutes, test products are extracted from
disinfectant solution, placed for 5-10 minutes. in solution
corresponding neutralizer. After washing, flushing liquid
0.1 ml are sown in 3-5 tubes with a slanting nutrient medium

to test the effectiveness of decontamination.

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The crops are kept in a thermostat at a temperature and time, optimal for growth of the used test-microorganism: for test-mycobacteria - 37 ± 1 ° C and 21 days, after which the registration is carried out results of the experiment.

AT quality control use test objects, contaminated as indicated above and placed in water at the time disinfection aging.

The multiplicity of the statement of the experiment must be sufficient for obtaining statistically reliable results.

The regime (concentration-time-temperature) is considered to be effective; Ensuring the death of the test microorganism on all test products in the absence of its growth in a nutrient medium. Positive A sample with a characteristic growth of a microorganism on a dense nutrient medium, or the detection of microorganisms in solution used neutralizer. If there are positive samples experiment repeated, increasing the exposure time, but not more than up to 60 min.

If necessary, the effectiveness of the developed mode is tested in practical conditions.

The criterion of decontamination efficiency is 100% microorganism.

The disinfection time is not more than 60 min.

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1.2.3.5. The study of tuberculocidal efficacy of DS, intended for the disinfection of linen, clothing, overalls and other objects from the fabric

Studies with DS are conducted to assess the effectiveness of its for decontaminating laundry and other objects from the fabric, clean and contaminated with blood or secretions (feces, urine, sputum).

Evaluation of the effectiveness of DS for disinfection of clothes, clothes, Work clothes and other objects from the fabric are carried out with the help of Sterile test objects, which are pieces of calico size 2 × 2 cm. Calico is pre-cooked and disinfected as well as a cambric. Sterile test objects are contaminated with a suspension of test-microorganisms containing $2 \cdot 10^9$ CFU / ml, at a rate of 20 ml for 10 test objects and dried for 30 minutes. Then the test-Objects are laid in coarse pouches of 5 × 8 cm size (2 pcs in each each), which is closed in the form of an envelope.

The solution of the tested DS on drinking water is room temperature temperature or heated to 50 ± 1 ° C is prepared at the rate of 5 liters per 1 kg linen. Rags, imitating linen, are individually poured into a container with solution of the tested DS so that between the layers of tissue is not formed air strata that impede the process disinfection. Simultaneously, between the layers of laundry are distributed (top, middle and bottom) pouches with contaminated test-objects. After a given time, the pouches are extracted simultaneously from three layers. Test objects are removed from the bag with sterile tweezers, is placed for 5 minutes. in a container with a solution of the corresponding neutralizer, then transferred to sterile drinking water and seeded on nutrient agar. The cultures are incubated at 37 ± 1 ° C. Preliminary recording of the results is carried out after 10-14 days, and

the final one - after 21 days. In control experiments, underwear immersed in sterile drinking water. Pouches with tests pawn in the same way as in the experiment. When obtaining a 100% death test-microorganism in experiments on the disinfection of laundry without protein contamination goes to experiments on the disinfection of laundry,

contaminated by secretions.

To determine the effectiveness of DS in the disinfection of laundry, clothes, overalls and other objects from tissue contaminated with blood, secretions (feces, sputum, urine, etc.), in the laboratory use coarse test objects that are contaminated with a suspension test-mycobacteria with the addition of 40% inactivated serum (6 ml of a suspension containing 2 × 10 ^{9} cfu / ml test microorganism mixed with 4 ml of inactivated whey) or 40% of fecal emulsion (6 ml of the suspension of the test microorganism are mixed with 4 ml of 40% fecal emulsion) based on a calculation of 30 ml of suspension on 10 test-objects. To prepare a fecal emulsion, 8 g of feces grinded in a mortar with 20 ml of water. The amount of test-A microorganism containing serum or feces is prepared from calculating 30 ml for 10 test objects. Contaminated test objects dried in a thermostat at 37 ± 1 ° C for 20-25 minutes. or 1.5-2 hours at room temperature until completely dry. Methodology The experiment is similar to experiments with clean linen.

Criterion for the effectiveness of DS in the disinfection of laundry, clothes, overalls and other objects from the fabric is 100% death of test microorganisms at test sites.

When studying the effectiveness of disinfection of products from synthetic fabrics (kapron, acetate, lavsan, etc.) use test-Objects from these tissues measuring 5 × 5 cm, because microorganisms are not

penetrate into the structure of these tissues and wash out them 2 times more, than with bazevyh test objects.

1.2.3.6. Research tuberculocidal efficacy chamber method of disinfection

The chamber method is used to disinfect clothes, shoes, bedding, soft toys, etc.

As a test microorganism, Mycobacterium B5 in in the form of a suspension containing 2 × 10 ^{9} cfu in 1 ml, which is contaminated test objects from cambric, coarse calico and other materials corresponding to disinfected objects. Test objects are laid in sterile

Envelopes from cotton fabric (2 test objects per envelope).
Numbered test objects are placed in cotton
Pouches with maximum thermometers and placed in the thickness
objects in the camera control points on three levels.

After disinfection, the bags are removed from the chamber and
record the readings of the maximum thermometers. Test objects
are placed in test tubes with 5 ml of potato-glycerin broth.

Incubation of crops with test objects is carried out at
temperature 37 ± 1 ° C for 5-7 days. Absence of turbidity
The nutrient medium indicates the death of mycobacteria in test objects.
In the presence of growth, a comparison of the isolated culture with the test-
mycobacterium.

As a control, test objects that are not
were placed in a chamber, and the nutrient medium used for
cultivation of the test microorganism after treatment. Control
Test objects and the environment are tested similarly to test objects that

was processed in a chamber. To establish the efficiency of processing
Perform at least three experiments for each treatment time.

Effectiveness of disinfection of things in disinfection
cells should be equal to 100% of death of Mycobacterium B5 by
used test objects.

1.2.3.7. Research tuberculocidal efficacy DS at disinfection of hands in rubber gloves

As a test microorganism, a suspension of M. terrae,
containing (1.0-5.0) · 10^8 cfu / ml, which is diluted to a content of 10^7 ,
10^5 and 10^3 cfu / ml.

To eliminate extraneous microflora of the hand, including wrists
and forearms, the testers carefully wash with soap in a warm flowing
water, then wipe with a sterile gauze napkin and put on
latex gloves.

The surface of rubber gloves worn on the hands of testers-

volunteers, are contaminated by thorough trituration of 1 ml suspension by the above three dilutions (each dilution at one test). After the microbial suspension for drying has dried initial contamination from the surface of the rubber gloves of the rear hand brush is done by washing with a sterile gauze napkin 5 × 5 cm, moistened with sterile drinking water. Then a napkin is placed in a test tube with 10 ml of sterile drinking water with beads and shake for 10 minutes. The resulting wash is seeded in 0.5 ml per the slanting surface of the culture medium in a test tube.

To disinfect the surface of gloves in the compressed palm of the hand In the glove, 2.5 ml of the test DS are applied to the volunteer test. Then he rubs this portion of disinfectant for 10-15 seconds

solution surface of the gloves of both hands, making movements of hands, which are performed with an antiseptic treatment of the skin of hands. After that the same operation is carried out, applying 2.5 ml of disinfectant solution on the palm of the second hand, and mark the stopwatch exposure.

After 5 minutes. (exposure) make flushing with a gauze napkin (5 × 5 cm), moistened relevant neutralizer, previously tested for neutralization efficiency and static action. The napkin is placed in a test tube with 10 ml sterile neutralizer for 10 min. Then the napkin is transferred Sterile tweezers into a test tube with 10 ml of sterile drinking water with beads, shake the tube for 10 minutes. in the shaker. From the obtained flushing is done by sowing 0.5 ml per solid nutrient medium in test tubes (not less than 3 test tubes per sample).

The mode of application of DS providing 100% death of the test microorganism on rubber gloves, which protect the skin of the hands.

1.2.3.8. The study of tuberculocidal efficacy of DS, intended for decontamination of secretions (urine, feces, sputum) and blood

Disinfection of urine.

The urine is poured into flasks or test tubes of 8 ml, add 1 ml suspension containing $2 \cdot 10^9$ CFU / ml M. terrae, and 1 ml inactivated horse serum.

The solutions of the tested DS are prepared in concentrations, tuberculocidal effect when tested for batistovyh test objects with protein protection.

Test solutions of DS are added to urine in equal or double volume. Note the contact time and at intervals 15, 30, 60 min. a mixture of 1 ml of pipette is transferred into tubes with 5 ml of the appropriate neutralizer. After careful mixing 1 ml of liquid from the first tube is transferred to the second test tube with 5 ml of neutralizer and then inoculated with 0.1 ml into tubes On the sloping surface of the nutrient medium, both from the first and from the second test tube. The crops are incubated in a thermostat at 37 ± 1 ° C.

Control is similarly performed experiments with only adding to the urine is not a disinfectant solution, but water.

An approximate recording of the results is carried out after 10-14 days, the final - 21 days. The results of the experiments are taken into account in attitude to control, which is taken as 100%. The final conclusion on the effectiveness of the DS are made on the basis of at least three experiments with coinciding results.

An effective means and mode of its application is considered effective, providing 100% death of test microorganisms.

Decontamination of feces. In the development of disinfection regimes feces take into account the ratio of DS to the disinfected mass, time processing, temperature, the consistency of disinfected emissions, degree of homogenization in the process of decontamination.

Research is carried out in two stages. At the first stage, as test-object use 20% emulsion of feces, on the second - decorated feces.

To prepare a 20% emulsion, 20 g of stool is ground in a mortar and add 80 ml of water; the resulting emulsion is filtered through double layer of gauze, sterilized in an autoclave, poured into a

test tubes of 9 ml and add 1 ml of 2 × 10 9 CFU / ml of suspension

M. terrae. Experiments are started with a concentration that causes death of test microorganism in urine with protein after 30 minutes. The prepared emulsion of feces is poured in equal or double volume of disinfectant solution and further produce seedings so the same as in the disinfection of urine. The results take into account in 21 days.

With positive results, experiments with large number of decorated feces (200-250 g). To do this, place them in a vessel, pour a disinfectant solution or fall asleep dry DS in equal or double amounts in relation to the weight of feces, determine whether the homogenization of feces. Then a small part of feces is stirred with a glass rod with a liquid, and The rest of the mass is left in the form of small lumps. Across certain time intervals (for example, 30, 60 minutes) are carried out sowing.

The sowing of the liquid part of the feces is carried out in the same way as in experiments with urine. Dense parts (lumps) are taken away by bacteriological loop and placed in 5 ml of the appropriate neutralizer, raster them on the edge of the tube and mix thoroughly. Then use a sterile pipette Transfer 1 ml of the mixture to the second test tube from this tube, also containing a neutralizer. Both from the first and from the second test tube 0.1 ml on a sloping surface of nutritious medium in test tubes (no less than three test tubes). Preliminary the result is taken into account after 10-14 days, and the final one through 21 days.

Control is similarly performed experiments with adding water instead of a disinfectant solution. results Experiments are taken into account with respect to control taken

for 100%. The effectiveness of the test substance is judged on the basis of at least three experiments with coincident results. Effective consider the means and mode of its application, providing 100% death of test microorganisms in the disinfected material.

Disinfection of blood and sputum.

As test objects in the evaluation of tuberculocidal effectiveness of DS, intended for blood disinfection, use blood, and sputum - chicken protein. To contaminate the test-objects with a test microorganism to 9 ml 40% blood or 50% chicken 1 ml of a suspension of the test microorganism containing $2 \cdot 10^9$ cfu / ml, mix and pour 1 ml into sterile bottles. Then they fall asleep or poured into the bottles to examine the DS in volume (5%, 10%, etc.) ratios to the material under study. At regular intervals (1 hour, 2 hours, etc.) with Using a sterile bacteriological loop, a selection is made samples of the mixture and transfer it to 5 ml of neutralizer for neutralization residual effect of the DS on the test microorganism. After 5 minutes. from this tube using a pipette, 0.2 ml of the test sample on the sloped surface of the nutrient medium in test tubes. Test tubes with inoculations were incubated at 37 ± 1 ° C.

The preliminary result of growth of test microorganisms on The cups are counted after 10-14 days, and the final one after 21 days.

An effective means of ensuring 100% death is considered effective test-microorganism in the disinfected material.

1.2.3.9. The study of tuberculocidal efficacy of DS, intended for the disinfection of medical waste

When studying tuberculocidal activity of DS with the aim of development of disinfection of medical waste use test objects from rubbers, plastics, textile materials,

glass, metals. For the preparation of test objects, sterile
disposable medical products (bandages, cotton
tampons, fragments of systems for blood transfusion and medicinal
preparations, catheters, spatulas, syringes, needle gloves, disposable
linen, napkins, pipettes, tubes, etc.) are ground and immersed in
a suspension of the test microorganism containing $2 \cdot 10^9$ CFU / ml
with the addition of 80% inactivated horse serum or
serum of cattle. After sufficient impregnation
Objects are removed into a dry sterile container and dried in a
thermostat at 37 ± 1 ° C for 20 min. or at room temperature
temperature 18-20 ° C and relative humidity 50-60% T
for 1 hour.

The contaminated test objects are immersed in a container with
tested disinfectant solution so that it is completely
closed them. Control of the effectiveness of disinfection of objects
spend every 15-30 minutes. for up to 360 min. For
this tests from different materials (two each name)
is removed from the disinfecting solution, washed in solution
corresponding neutralizer, from the produced washings are produced
sowing 0.1 ml on the surface of a solid nutrient medium.
Control test objects are immersed in sterile tap water
water for the period of maximum exposure, and then sow on
sloping surface of the nutrient medium. Test tubes with crops

is placed in a thermostat at a temperature of 37 ± 1 ° C. Final accounting
the results are carried out for 21 days.

Criterion of effectiveness of decontamination of medical waste
- 100% death of M. terrae on test objects treated with DS.

1.2.3.10. Methods for determining tuberculocidal activity of DS at disinfection of air

Chemical DS are used for air disinfection
in premises in the form of aerosols or vapors of DS solutions, as well as gases.

When investigating the effectiveness of air disinfection

chemical DS with the aim of developing the regime of its application at
tuberculosis as test micro-organisms use M. terrae.

Studies are carried out in test chambers with a volume of
1 or 2 m^3 . Preliminary inner surface of the chamber is washed
detergent solution, the residues of which are then washed off
tap water and include a bactericidal UV irradiator. AT
center of the chamber have a disinfected fan,
productivity of 15-25 m^3 / hour, the purpose of which -
prevention of rapid subsidence of microorganisms.

To determine the effectiveness of air disinfection
use aspiration method.

In the chamber, the suspension of the test microorganism is sprayed into
sufficient to create a concentration chamber in the air
microorganisms 2,1x10^4 CFU / m^3 (determined experimentally).

The aerosol of the disinfectant is created by
spray equipment, which provides the formation of
air of at least 80% of particles with a dispersion of 10-15 μm, and include
fan. Then a solution of the test substance is sprayed into the chamber and

at certain intervals check the dissemination
air.

The aspiration method is based on air aspiration through
liquid. When using the aspiration method for evaluation
The effectiveness of air disinfection requires the following
equipment:

– blower with a capacity of 15-25 l / min;

– sterile Drexel flasks with 50 ml of sterile
tap water at the rate of 2 pcs. for 1 trial. Preliminary in
Sterile water is introduced by a neutralizer, corresponding to the subject
DS;

– α tube with a diameter of 8-10 mm, a length of 50-60 cm, which is introduced in
chamber for sampling air in the center of the chamber;

– sterile rubber hoses connecting Drexel's flasks
(successively one after another) and then with a blower.

Preparing the camera for the experiment is carried out as indicated higher. 50 liters of air (volume of the sample) are taken for the study.

The sampling sequence is as follows:

1 - control of air contamination before spraying of the suspension of test microorganisms;

2 - control of air pollution after spraying the test culture slurry;

3 - control of the effectiveness of air disinfection

- Sampling every 5-10 minutes. depending on the effectiveness of DS.

After sampling, the liquid from the 2 Drexel bottles is mixed and 1 ml are introduced into test tubes with a slanting nutrient medium. Crops is placed in a thermostat. Preliminary recording of results

after 10-14 days, the final - after 21 days. Criterion efficiency of air disinfection - 100%.

1.2.4. Methods of investigation and evaluation of tuberculocidal activity of DS in practical terms

Practical tests are carried out in cases where DS contains a new active substance and requires confirmation The efficiency of the regimes developed in the laboratory the causative agents of tuberculosis and mycobacteriosis.

The tests are carried out in accordance with the Test Instruction DS, assessing tuberculocidal efficacy, safety application and reliability of recommended precautions, physical and chemical properties of DS: solubility, odor, presence detergent action, etc.

When evaluating the effectiveness of the DS, seeding objects the surrounding environment, having epidemiological significance in tuberculosis, before disinfection (background) and compare it with the contamination of these objects after treatment with a working solution of DS.

An effective means is considered, after treatment in which in accordance with the regime specified in the Instruction,

they detect pathogens of tuberculosis, mycobacteriosis and S. aureus.

Upon completion of the tests, a test report is issued, in which the above parameters and other marked properties are reflected.

1.2.5. Apps

Annex 1 (informative)

Characteristic test-mycobacteria by morphological, cultural and biochemical properties.

Mycobacterium AT 5 present by yourself polymorphic, fixed, non-spore-forming sticks, 0.2-0.6x1-10 microns in size (as well as other types of mycobacteria).

Mycobacterium B5 - Gram-positive, aerobes, grow on Electroactive nutrient media on an egg basis (the reaction of the environment is almost neutral (pH 6.8-7.2)): Petranjani, Levenstein-Jensen, "New" and others at an optimum temperature of 37 ° C, although they can grow at temperature 25-38°C. Colonies of Mycobacterium B5 on dense nutrient media are rough, matte,
The convex formations are bright orange-yellow in color. Orange-yellow coloration of the colonies is due to the pigment, which is formed when cultivation of Mycobacterium B5 in the dark. Duration of growth Mycobacterium B5 on nutrient media is from 5 to 7 days. On elective synthetic nutrient media (for example, Middlebrook 7N9 with 10% growth additive ADC, 7N10 with 10% growth additives OADC) Mycobacterium B5 grows poorly or does not grow at all.

In smears from young cultures Mycobacterium B5 have the form short, thick sticks, located one by one. In the old cultures are ovoid, columbiform or fusiform shape. They are colored according to Tsil-Nielsen in crimson-red color.

Mycobacterium B5 show a more pronounced catalase

activity than the virulent species of mycobacteria, and to a greater extent, than the latter, decompose hydrogen peroxide. Peroxidase

Mycobacterium B5 is more resistant to temperature effects than such as virulent mycobacteria. Mycobacterium B5 is not coagulated milk, do not liquefy gelatin.

By classification of pathogenic microorganisms for humans Mycobacterium B5, like all mycobacteria, belong to group IV are non-pathogenic microorganisms.

Mycobacterium B5 is used to assess the effectiveness of chamber disinfection of things, clothes and other objects as most adequate model causative agent tuberculosis to the temperature effect.

The test microorganism Mycobacterium B5 is stored in the museum of the FGUN "Research institute disinfectology » Rospotrebnadzor (117246, Moscow, Nauchny proezd, 18).

Mycobacterium terrae (ATCC 15755, DSM 43227) represent a short straight sticks of crimson-red color, located in the smear in parallel to each other, like stockade. Mycobacteria M. terrae are immobile, Gram-positive, not forming spores and capsules. Due to the high content Mycolic acids are poorly colored by conventional methods, but they are well colored according to Tsil-Nielsen in crimson-red color.

Mycobacteria M. terrae - aerobes, temperature boundaries of growth - from 25 ° C to 37 ° C, the optimum temperature is 37 ° C.

M. terrae are demanding of nutrient media, grow on elective media (the reaction of the environment is almost neutral (pH 6.4-7.0): Levenstein-Jensen, "New", Middlebrook 7N9 with 10% growth additives ADC, 7N10 with 10% growth supplement OADC, etc. Colonies M. terrae on a Lowenstein-Jensen medium - rough and matte convex rounded formations, on the medium "New" - smooth and

shiny or with a milky or yellowish tinge. Duration
growth of M. terrae on nutrient media depends on the composition of the medium. When
optimal temperature of incubation on "rich" nutrient
Levenstein-Jensen environments and the "New" growth of M. terrae may appear
for 5-7 days. On "hungry" nutrient media, incubation of M. terrae
is up to 8 weeks.

M. terrae hydrolyze Tween 80 (because they produce lipases), have
highly active catalase and exhibit β-galactase activity.
Restore potassium tellurite to metallic tellurium for
9 days.

By classification of pathogenic microorganisms for humans
refers to the IV group of danger, is non-pathogenic
microorganism.

Annex 2 (recommended)

Methods of preparing nutrient media for the cultivation of test-
Mycobacteriums for study and evaluation
tuberculocidal activity of DS and their substances.

Method of cooking medium Petranjani

The following components are included in the formulation of the nutrient medium:

peptone - 2 g;

starch - 12 g;

milk - 300 ml;

eggs - 10 pieces;

potatoes - 2 pieces;

malachite green - 2 g;

glycerin - 24 ml;

water 100 ml.

12 g of starch and 2 g of peptone are placed in a sterile flask (1 L).
Then add 2 pcs. finely chopped potatoes (potatoes
previously washed with soap and a brush, clean). Add to flask
300 ml of fresh whole milk and heated on a water bath in

for an hour (from the moment of the beginning of coagulation of milk) at a constant stirring. In another sterile flask, 8 dietary eggs and 2 yolks. Fresh dietary chicken eggs with a shelf life Not more than 7 days without cracks and shell defects thoroughly washed in warm running water with the help of hand brushes and alkaline soap, then left for 30 minutes. in a soap solution. Rinse thoroughly in running water and immersed in 70% ethyl alcohol for 30 minutes. Before how to start working with clean and dry eggs, it is recommended that Wash hands thoroughly with soap and water. Eggs are thoroughly beaten to a homogeneous mass and poured into a flask with a mixture of starch, peptone, potato and milk. Add 24 ml of glycerol and 16 ml of 2% solution of malachite green. The resulting mixture carefully mix, filter through a sterile gauze filter in a sterile flask. For the coagulation of the medium, special devices-convolutors of type "ASPS". Test tubes with spilled in them the medium is placed in special tripods with a selected angle tilt to form a beveled surface of medium height 8-10 cm. The tripods are mounted in a convolver and conducted Coagulation at 85 ° C for 45 min.

Preparation of malachite green solution:

Malachite green - 2 g;

Sterile distilled water - 100 ml.

Suspended powder of malachite green is dissolved in sterile warm distilled water and place the solution in

thermostat for 1-2-2.5 hours for greater dissolution. Then filtered solution through a paper filter, poured into bottles or small flasks and sterilized in a steam sterilizer at 1 atm. (121 ° C) for 30 minutes. The prepared solution is not subject to long-term storage, and when precipitation or discolouration occurs it is replaced with fresh solution.

Method of preparation of potato-glycerin broth.

Prepare a potato broth. For 1 liter of drinking water take 200g peeled (potatoes are prewashed with soap and a brush,

clean) the potatoes, boil for 30 minutes from the time of boiling,
cool and defend. Then filtered through a cotton-gauze filter
and adjusted to the original volume with water, 10 ml
glycerin. The pH of the potato broth is 7.0-7.2. Broth is sterilized in
steam sterilizer at 1 atm. (1210C) for 30 minutes.

Method of preparation of potato-glycerin agar

The following components are included in the formulation of the nutrient medium:

Potato-glycerin broth;

Peptone - 5 g;

Mel - 1 g;

Agar-agar - 20 g;

Water - 1 liter.

To a potato-glycerin broth add 5 g of peptone,
20 g of agar-agar, 1 g of chalk, set the pH to 7.0-7.2. Agar is melted and
poured into test tubes or flasks. Sterilized in steam
sterilizer at 1 atm. (121 ° C) for 30 minutes.

Method of preparation medium Middlebrook 7H9 (broth)
with 10% ADC growth additive (MADC-broth for storage
frozen cultures at minus 70 ° C)

Middlebrook 7H9 broth powder - 4.7 g;

Glycerol (C3H8O3) - 100.0 ml;

Water (sterile, distilled) - 750,0 ml.

Ingredients combine, mix, sterilize in steam
sterilizer at 1.1 atm. (121 ° C) for 10 minutes, cool to 45 ° C.
Add under aseptic conditions 100 ml of ADC and
sterile distilled water to 1000.0 ml.

The pH of the medium will be equivalent to 6.6 ± 0.2 in the broth cooled to
25ºC.

The method of preparation of the Levenstein-Jensen medium

The Levenstein-Jensen environment is an international environment, widely

Used as a standard medium for primary isolation
causative agent of tuberculosis and its drug

sensitivity.

It is a dense egg environment on which the visible growth of M. terrae

are obtained approximately 7-10 days after sowing. For cleanliness

It is desirable to use the prepared nutrient medium in

standard test tubes with screw caps. When

The impossibility of purchasing a ready-made environment, it can be prepared

yourself.

The composition of the Levenstein-Jensen environment includes the following

Components:

Solution of mineral salts:

Potassium monosubstituted phosphate - 2.4 g;

Magnesium citrate - 0.6 g;

Magnesium sulphate 0.24 g;

L-asparagine - 3.6 g;

Glycerol 12.0 ml;

Distilled water - 600 ml.

The above ingredients are dissolved in warm

distilled water in the indicated sequence with a weak

heating (without boiling) in a water bath. L-asparagine

it is recommended to dissolve separately and make the last. Then

The saline solution is sterilized in a steam sterilizer at 1 atm.

(121 ° C) for 20-30 minutes. The storage life of the solution is

3-4 weeks at room temperature.

Solution of malachite green:

Malachite green - 2 g;

Sterile distilled water - 100 ml.

Suspended powder of malachite green is dissolved in

sterile warm distilled water and place the solution in

thermostat for 1-2-2.5 hours for greater dissolution. Then filtered

solution through a paper filter, poured into bottles or

small flasks and sterilized at 1 atm. (121 ° C) for

30 min. The prepared solution is not subject to long-term storage,

and when a deposit or color change occurs, it is replaced with fresh solution.

Egg mass.

Fresh dietary chicken eggs with a shelf life Not more than 7 days without cracks and shell defects thoroughly washed in warm running water with the help of hand brushes and alkaline soap, then left for 30 minutes. in a soap solution. Rinse thoroughly in running water and immersed in 70% ethyl alcohol for 30 minutes. Before

how to start working with clean and dry eggs, it is recommended that Wash hands thoroughly with soap and water. Then in a sterile box break eggs with a sterile knife in a sterile dish, leading a common the volume of egg mass to 1 liter (this requires an average of 20-25 eggs, depending on their size). Whisk the egg mass sterile corolla or in a sterile mixer with minimal speed.

Preparation of the medium.

In a large sterile container, observing the rules of asepsis, the following solutions are placed:

A solution of mineral salts - 600 ml;

The homogenized egg mass is 1000 ml.

The mixture is thoroughly mixed and filtered through a 4-layer sterile gauze filter. Add 20 ml of a solution of malachite green, thoroughly mixed, avoiding the formation of foam, and in for no more than 15 minutes. is poured into test tubes approximately 5 ml, making sure that no precipitate forms in the solution.

Coagulation (coagulation) of the medium.

For the coagulation of the medium, special apparatus-curlers of the "ASPS" type. Test tubes with a spill in them are placed in special stands with a selected angle of inclination for forming a beveled surface of a medium 8-10 cm high. The tripods are placed in the coagulant and coagulated 85 ° C for 45 min.

Storage medium.

The final nutrient medium is checked for sterility, placing 10 tubes from the newly prepared batch to a thermostat at 37 ° C for 3 days. After the incubation time in test tubes at

the microbial growth should be absent from the nutrient medium. When The availability of growth of the prepared batch of medium is subject to destruction. A prepared batch of medium must have a label with a date and stored in a refrigerator at 4 ° C with care closed stoppers to prevent drying. Shelf life environment should not exceed 4 weeks.

Method of preparation of the environment "New"

The "New" environment is a dense egg environment, on which a good the growth of M. terrae colonies is about 5-7 days after sowing of the material (samples).

The formulation of the Nova nutrient medium includes the following components (in% by weight):

Potassium phosphate monobasic	0.05
Sodium citrate	0.05
Magnesium sulphate	0.05
Sodium pyruvic acid (glycine)	0.2
Glycerol	3.6
Malachite greenery	0.036
Yolks of eggs	50.0
Distilled water	up to 100,0

A method for producing a concentrated nutrient medium is to mix dry additives of salt ingredients (except for bactericidal dye) with yolk mass, sterilization ingredients with 70% ethyl alcohol, dissolving bactericidal dye in glycerin and the connection of all components of the medium.

To obtain 1 liter of concentrated culture medium you need:

make the sample: single-substituted phosphoric acid potassium - 1 g, sodium citrate - 1 g, magnesium sulfate - 1 g, sodium

pyruvic acid (glycocal) - 4 grams, and place each hinge in a sterile porcelain mortar;

disinfect the sample by wetting each sample with 1 ml 70% ethyl alcohol, then place the mortar with the hinges in the thermostat at a temperature of 37 ° C and dry with periodic stirring in for 45 minutes;

prepare 1 liter of yolk mass: 50 pieces of chicken eggs To treat outside with the purpose of disinfection with 70% alcohol, then Separate the protein from the yolk, transfer the yolk mass to a volumetric flask and thoroughly homogenize.

Next, prepare the yolk-salt mixture: to 1 liter of vitelline Add the disinfected samples and mix.

Prepare a solution of bactericidal dye: 700 mg sample malachite greens to moisten 1 ml of 70% alcohol and combine with 70 ml glycerin (this corresponds to a 1% solution of malachite green in glycerol).

Preparation of concentrated nutrient mixture.

A compound of 1 liter of a yolk-salt mixture with 70 ml 1% solution of malachite green in glycerin and carefully homogenize. The resulting concentrated nutrient mixture (medium) is kept for 24 hours at room temperature and pour the medium into 250 ml vials that are sealed pack and mark. Sealed bottles from the concentrated nutrient medium is stored in a refrigerator at 3-5 ° C for 3-4 weeks.

Preparation of the working (used for testing DS) dense nutrient medium in test tubes.

An equal volume is added to the concentrated nutrient medium sterile distilled water (1: 1), homogenized, poured 5 ml in tubes. To form the bevel of the nutrient medium

the tubes are laid in an inclined position in a convolver,

preheated to 90 ° C. The medium is coagulated for 20 minutes. at

temperature 82-83 ° C. Control of the prepared batch of medium for

Sterility is also carried out as described for the medium

Levenshtein-Jensen.

Tubes with nutrient medium can be stored in the refrigerator

at a temperature of 5 ° C for 4 weeks. With long-term storage

tubes should be tightly sealed and placed in a

Polyethylene bags to prevent drying out of the environment.

Annex 3 (recommended)

Materials and equipment needed to study
tuberculocidal activity of DS

Chemical substances:

magnesium sulphate $MgSO_4 \times 7H_2O$ - GOST 4523-77;

sodium citrate $C_6H_5O_7Na_3 \times 5.5\ H_2O$ - GOST 22280-86;

potassium phosphate monosubstituted KH_2PO_4 - TU-6-09-5324-87;

sodium pyruvic acid - TU 6-09-08-990-83;

glycerol $C_3H_8O_3$ - GOST 6259-75;

malachite green $C_{52}H_{54}O_{12}N_4$ - TU-6-09-1557-77;

distilled water - GOST 6709-77;

yolks of chicken eggs;

magnesium citrate $Mg_3(C_6H_5O_7)_2 \times 14\ H_2O$ - TU-6-09-1770-77;

L-asparagine $C_4H_8N_2O_3 \times H_2O$ - imported reagent;

Middlebrook 7N9 broth powder - import medium;

10% ADC growth additive - imported reagent;

neutralizing broth Di-Ingli - firm HIMEDIA;

alcohol ethyl, rectifying technical - GOST 18300-72;

sterile distilled water.

Equipment:

Laboratory scales (for example, scales for chemical reagents with

accuracy to 0.01 g, GOST 24104-80, recommended for use
Order of the Ministry of Health of the Russian Federation No. 109);

apparatus for clotting nutrient media (eg, ASPS-

Torgmash, reg. No. 29/07020401 / 3573-02);

thermostat at 37 ° C (for example, the thermostat is dry-air

TV-80- "PC-K", reg. No.98 / 219-151);

low temperature freezer storage

test cultures of microorganisms (for example, an ultra-low freezer

temperatures MDF-4086S, reg. No. 60202380);

camera for 1 or 2 m^3 with the necessary

study of air disinfection;

microscope;

aerosol generator;

hydropulse, automatic, "Quasar";

shaker (for example, laboratory shaker S-3.02, registration No. 1441

GOST 12.2.05.);

household refrigerator (for example, fridge-freezer

STINOL 256Q Ser. No. 103410881041);

electric household cooker, GOST 14919-83;

laboratory chemical stands, TU 48-0534-8-87;

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test objects: cloth, coarse and cambric; surface size

10x10 cm from various materials; subjects of care for patients;

toys are small and large of various materials; medical

tools are diverse in form, surface and

material; rigid and flexible endoscopes; a set of rubber and

plastic tubes; tableware and appliances; glassware

and utensils from under the discharge; bandages, cotton wool;

stopwatch mechanical, GOST 8.423-81;

thermometers, GOST 215-73;

a pencil on glass, TU 46-22-904-78;

glassware:

pipettes with a capacity of 1.0 and 10.0 cm^3 2nd class of accuracy, GOST

20292-74;

chemical test tubes, GOST 10515-6;

Petri dishes, GOST 25336-82;

Laboratory alcohol, GOST 25336-82 or gas burner;

cylinders with a capacity of 100.0 and 250.0 cm^3, GOST 1770-74;

flasks with a capacity of 100.0; 250.0 and 1000.0 cm^3 of GOST 25 336-82;

flasks of Drexel.

1.3 Methods for studying and evaluating fungicidal activity disinfectants

1.3.1 General

1.3.1.1 Test microorganisms for studying fungicidal activity of DS and their substances. Requirements for test fungi

When studying fungicidal activity of DS and their substances in Fungi cultures are used as test microorganisms:

Candida albicans (strain 15), Candida albicans ATCC 10231 (DSM 1386) for the evaluation of fungicidal activity against pathogens candidiasis,

Trichophyton gypseum (music strain NIID), Trichophyton mentagrophytes ATCC 3533 (DSM 4870) - for evaluation of fungicidal activity against pathogens of dermatophytosis,

Aspergillus niger (muscular strain NIID), Aspergilla niger ATCC16404 (DSM 1988) for the evaluation of fungicidal activity against mold fungi of the genus Aspergillus.

At certification tests and an expert estimation earlier registered DS, a set of test microorganisms may be

It is limited to the most stable representatives of each group.

Conditions for cultivation of test fungi.

Test microorganisms are cultured on the following nutrient environments:

C.albicans - on Saburo broth, Saburo agar at temperature 27 ± 1 ° C for 2-10 days or at a temperature of 30 ± 1 ° C within 2-5 days;

T.gypseum - on Saburo broth, Saburo agar at temperature 27 ± 1 ° C for 28 days;

A. niger - on Saburo broth, Saburo agar at 37 ° C for 2 days, and then stand for 3-5 days at a temperature of 20 ± 2 ° C in a dark place.

Museum cultures of the above microorganisms store at a temperature of 3 ± 1 ° C in ampoules (after freeze-drying) or dense nutrient media (seeding with a prick) under a layer of sterile vaseline oil (the thickness of the layer is 1.5-3 mm), and the working crops are chopped agar or in broth.

Test microorganisms should have typical biochemical, morphological, tinctorial, cultural and enzymatic properties inherent in this species and have a standard resistance to reference DS: solutions of chloramine, peroxide hydrogen, Catamine AB - alkyl dimethylbenzylammonium chloride (ADBAH), glutaraldehyde (Table 5.7.).

Table 5.7

Stability of test microorganisms for disinfectants

Disinfectant means	Concentration solution on DV,%	Time of death of test fungi, min. not less than		
		C.albicans	T.gypseum	A.niger
Chloramine	1.0 *	35	50	-
ADBAH	0.5	5	25	-
Glutar aldehyde	1.0	15	-	-
	2.0	-	thirty	-
	2.5	-	-	60
Hydrogen peroxide	4.0	-	50	-
	6.0	> 60	-	60

Note - the sign (*) indicates that the concentration of the solution is indicated by the preparation.

Methods of preparation of a suspension of test microorganisms. Definition biological concentration of test fungi in suspension.

Cultures of test fungi are subjected to control of their quality. AT particular, immediately before the use of crops for research objectives, you need to make sure that the test-strains grown on a nutrient medium are not contaminated by extraneous microflora. To assess the growth of cultures of test fungi visually scan each tube and take into account the nature and massiveness growth, change in color of the nutrient medium.

Working suspensions of test fungi are prepared from the culture of this test-strain grown on a nutrient medium.

The culture of T.gypseum, grown on Saburo broth at temperature 27 ± 1 ° C for 28 days, remove the loop from the tube, is placed in a porcelain mortar and rubbed with a small amount sterile physiological solution to a homogeneous suspension with the minimum particle size. The resulting suspension is filtered through a sterile cotton-gauze filter and adjusted with physiological solution according to the optical turbidity standard (produced by FGBU "NTSESMP" of the Ministry of Health of Russia, standard turbidity of McFarland) $2 \cdot 10^9$ microbial bodies in 1 ml. When lack of a ready-made turbidity standard The microbial bodies are carried out using a densitometer.

The culture of C. albicans is grown on Saburo agar for 2 days at a temperature of 27 ± 1 ° C. Then it is washed off with a nutritious medium with a small amount of sterile physiological solution and mix thoroughly. The resulting suspension is filtered and bred in the same way as the culture of T.gypseum.

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The A. niger culture, grown on Saburo broth for
2 days and aged for 3-5 days in a dark place, remove
loop from a test tube, placed in a porcelain mortar and rubbed with
with a small amount of sterile saline until
a homogeneous suspension with a minimum particle size. The received
the slurry is filtered through a sterile cotton-gauze filter and adjusted
to a concentration of $2 \cdot 10^9$ cfu / ml according to the optical turbidity standard
(produced by FGBU "NTSESMP" of the Ministry of Health of Russia, standard
turbidity of McFarland) $2 \cdot 10^9$ microbial bodies in 1 ml. When
lack of a ready-made turbidity standard
The microbial bodies are carried out using a densitometer.

Due to the fact that the suspension can contain, along with live
dead microorganisms, it is necessary to determine the biological
concentration of test fungi. For this, tenfold
dilution of the test-fungus suspension in sterile drinking water with
subsequent suspension of the suspension in a petri dish with a dense
nutrient medium (agar Saburo). After a certain time
The incubation at the appropriate temperature is counted
The number of grown colony-forming units (CFU)
the number of viable cells in one ml of the suspension.

The stability of test fungi to solutions of reference DSs is determined
by the method of cambric test objects (1.3.2.2). Stability test
spend at least 1 time per month. With a decrease in resistance of crops
make them re-enter into enriched nutrient media
before restoring stability.

Preparation of working solutions of DS and their substances is carried out in
according to clause 1.1.1.2.

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1.3.2 Methods of research and evaluation of fungicidal
activity of DS and their substances in vitro

The aim of the research is to determine the level and spectrum fungicidal activity of DS and their substances.

Substances (active substances) intended for production of DS must meet the following requirements:

dissolve well in water or other solvents;

fungicidal action, that is, to kill mushrooms, and not to delay their growth;

have satisfactory organoleptic (in color, smell) and physico-chemical (by solubility, biodegradability and storage stability, etc.).

The fungicidal activity of DS and their substances is determined by suspension method or the method of cambric test objects.

1.3.2.1. Suspension method

For the preparation of solutions of DS in various concentrations of DV means diluted or dissolved in sterile drinking water and 4.5 ml is poured into sterile test tubes. Add 0.5 ml suspension of the test fungus (see paragraph 1.3.1.1.) containing $1 \cdot 10^9$ cfu / ml, and mix thoroughly. At regular intervals (15 min.) For 0.5 ml of the "test-microorganism + DV" suspension is added to 4.5 ml of the appropriate neutralizer. Stir thoroughly and leave for 5 minutes. Then, 0.5 ml is added to a test tube with 4.5 ml sterile drinking water, after which 0.1 ml of this sample is added to the test tubes with 5 ml of liquid and on the surface of a solid nutrient medium. In control experiments, instead of DV, a sterile drinking water, and crops are made in an environment without a neutralizer and with a neutralizer.

Temperature of incubation of crops in thermostat and timing of registration the results of the experiment depend on the type of microorganism (clause 1.3.1.1). For confirmation of the removal of biocidal action of DV from test tubes in which there was no growth of test fungi, every day they do a 0.5 ml in 4.5 ml of a new culture medium.

The results of the experiment are assessed by the presence or absence of growth fungi in a liquid and on a solid nutrient medium. Comparison is carried out with

control of experience, which is the seeding of test fungi in a nutritious medium without addition of active substance.

Effective is the concentration of the agent, in which three times repeated experiment at a certain time of action gives negative result (no growth of fungi) if available typical growth of the test fungus in the control .

1.3.2.2. The method of cambric test objects

Preparation of the suspension of fungi is carried out in accordance with with clause 1.3.1.1. Preparation of cambric test-objects, their contamination and The experiment is performed as described in 1.1.2.2.

Store the contaminated test objects in Petri dishes in refrigerator at a temperature of plus 4 ° C.

The shelf life of test items contaminated with C.albicans - 4 days, T.gypseum and A.niger - 30 days.

The criterion for the activity of DV (substance) is 100% death test-fungi (no growth in test samples) with time disinfection: C.albicans, T.gypseum, A.niger - no more than 60 min.

1.3.2.3 Methods for eliminating fungistatic action active substances

To determine fungicidal and exclude fungistatic The actions of DV after the expiration of the exposure must be stopped influence of DV on the test culture. This is achieved by using following methods:

the use of a chemical neutralizer;

sowing in a large volume of nutrient medium;

daily re-entering new nutrient media.

The use of a chemical neutralizer, the control of it effectiveness and completeness of neutralization of DV during exposure to cultures of fungi are carried out in accordance with 1.1.2.3.

Criteria for the selection of active substances (substances). Criterion

selection of the active substance as a substance for the creation of the DS,
intended for disinfection of objects contaminated with
test fungi, is the presence of fungicidal activity.

The death of test microorganisms should be 100% at
duration of action (min.) DS in minimum concentration
in a relationship:

fungi C.albicans, T.gypseum - not more than 60 min .;

A.niger - not more than 120 min.

1.3.2.4 Methods for studying factors affecting fungicidal activity of DS and their substances

Studies include the determination of the fungicidal spectrum
actions of the DS, and in the course of in-depth study additional
determine the influence of various factors (pH, temperature,
organic substances) on the fungicidal activity of solutions of DS.

The study of the spectrum of fungicidal activity and the effect on
the activity of various factors is carried out by the method of cambric
test objects contaminated with test cultures C.albicans,
T.gypseum, A.niger.

A study of the dependence of the activity of DS on the presence
organic substances and pH, as well as studying the effect of temperature on
the activity of the DS under investigation is carried out in accordance with 1.1.2.4.

Criteria for evaluating the fungicidal activity of DS.

For DS, intended for disinfection of various
objects, the death of test fungi should be 100% with time
the action (min) of the disinfectant solution in the minimum
concentration of DW in relation to:

fungi C.albicans, T.gypseum - not more than 60 min;

A.niger - not more than 120 min.

The influence of environmental factors on the activity of DS is taken into account when
the development of optimal regimes and the application of DS in practice.

1.3.3. Methods of studying the fungicidal efficacy of DS, intended for disinfection of various objects of the external

medium contaminated with test fungi

The purpose of the study is the development of regimes for the use of DS with taking into account the conditions for their further application in practice for disinfection of medical devices, subjects of care of patients, toys, linen, surfaces, utensils, secretions, etc., depending on the type contamination, concentration of active substance, time impact, the rate of consumption, the nature of the object, the presence on it organic pollution and its specificity, temperature, method and fold processing.

1.3.3.1 Study of fungicidal effectiveness of DS, intended for the disinfection of medical devices

When choosing a DS for study with a view to the subsequent recommendation for disinfection of medical devices should be considered:

purpose and multiplicity of application of the product (multiple or single application);

Availability the facilities negative properties, eg, corrosive or fixing action, etc., limiting Possibilities of its use or requiring individual methodical approach to research;

material (s) from which the product is made;

functional features of the product and its conditions operation, which determine the specifics of the methodology experiment and subsequent recommendations on their technology. disinfection.

Determining the effectiveness of DS when processing medical products from various materials (except endoscopes).

As test products use sterile tools and medical devices (catheters, micropipettes, plastic spatulas, etc.) from various materials (metals, rubber, glass, plastic) or simulating their test objects. Scroll tools taken in the experiment should include at least three

tools with lock parts (forceps, scissors, corncang)
and at least two, without any lock parts (tweezers, spatulas),

as well as dental, including rotating, tools

(not less than 4) - boron, drilling root, mirror, disk grinding.

As test products from rubber, glass, plastics are used

fragments of products (catheters, micropipettes, spatulas, etc.).

As test fungi, suspensions of cultures of C. albicans,

T.gypseum. On the surface of the test product (in the case of

The area of the lock, and in the presence of channels and cavities - also in the channel

products) with a pipette, 0.1 ml of a 1 billion suspension of

or other species test microorganisms, containing 40%

inactivated horse serum. Test-products are dried

until completely dry. Small test products are immersed in said

suspension of fungi for 15 minutes, then they are removed and dried

(until completely dry). Disinfectant solutions are prepared for

sterile drinking water.

After drying, the contaminated products are completely

immersed in the solution of the tested DS, filling it with all channels and

The cavity of the products, avoiding the formation of air congestion.

Tools with lock parts are immersed open,

Preliminarily making them in the DS solution several working movements

for better penetration of the solution into hard-to-reach areas

products in the castle area. Thickness of a layer of DS solution over products

should be not less than 1 cm. In parallel for product control

immersed in water.

After a certain time (from 5 to 120 minutes), the products are removed from the

solution and a gauze napkin measuring 5×5 cm^2, impregnated

neutralizer, flushes are made from the surface of the product, then

the tissue is placed in a test tube with 10 ml of the same neutralizer and

shake with beads for 5-10 minutes. The product channel is washed

neutralizer solution. Small articles are immersed in a solution

neutralizer for 5 minutes, and then transferred to tubes with a liquid

nutrient medium. To control the effectiveness of disinfection flushing fluid from the surface of the article and from the channel are sown on the appropriate nutrient media (Section 1.3.1.1.). Crops are kept in a thermostat at a temperature and time, optimal for growth of the used test microorganism.

The multiplicity of the statement of the experiment must be sufficient for obtaining statistically reliable results.

The regime (concentration-time-temperature) is considered to be effective; ensuring the death of test fungi on all products. In the presence of positive samples experiment repeat, increasing the concentration or time of exposure.

Criterion of decontamination efficiency - 100% death C.albicans, T.gypseum.

The disinfection time of products contaminated with C. albicans, T.gypseum - not more than 120 min.

1.3.3.2 Study of fungicidal effectiveness of DS, intended for disinfection of endoscopes, including high-level disinfection

To determine the effectiveness of DS in disinfection endoscopes as test objects use sterile fragments of endoscope or endoscope (flexible - gastroscopy, hard - cystoscope), and as a test fungus - C. albicans.

On the external surface of the test object, 0.1 ml. suspension of C. albicans containing 5% serum; through the channel endoscope using a pipette let at least 5 ml of the same suspension. After that, the endoscope is dried for 20 minutes. Then the contaminated product is immersed in the DS solution, filling the cavities

and endoscope channels. At regular intervals during the

5-60 min. the product is removed from the solution and flushed from the outside surfaces gauze napkin, moistened at solution neutralizer. The product channel is washed with a neutralizer. Flushing the liquid is inoculated into appropriate nutrient media.

The criterion for the effectiveness of endoscope disinfection is 100% death of C.albicans no more than 60 min.

Determination of the effectiveness of DS in high-level disinfection level (TLD) endoscopes. This technique is designed for development of high-level disinfection regimens (TLDs) of endoscopes when conducting "non-sterile" diagnostic and therapeutic manipulations.

The DS intended for endoscope TLDs should provide death on endoscopes of C.albicans.

For the study, a DS effective for sterilization endoscopes, and it is tested under the same conditions (concentration, temperature) as in the sterilization of endoscopes, determining the necessary disinfection time for achievements doom test-microorganism. When determining the effectiveness of means for TLD endoscopes as test objects use fragments of a flexible endoscope channel or its imitators in The form of tubes made of plastic 2 cm long, 2 mm in diameter.

Sterile test objects are artificially contaminated, Using the dispenser / micropipette to the central part of the channel and the surface of each tube is a suspension of C. albicans at a rate of 106 cells C.albicans for each product. To simulate organic pollution to a suspension of microorganisms before contamination of the object is added 2% inactivated horse serum.

The contaminated test objects are dried at room temperature temperature (plus 18-22 ° C) for 20 minutes.

Disinfectant solution is prepared on sterile drinking water according to clause 1.2. Processing of the DS under investigation is carried out by the method immersion in the test disinfectant solution. Across certain time intervals, depending on the chemical composition means, in the interval from 5 to 60 minutes, test products are extracted from

disinfectant solution, placed for 5-10 minutes. in solution
corresponding neutralizer.

The test products are then placed in test tubes with a culture medium
for testing the effectiveness of disinfection: for C. albicans - broth
Saburo.

The crops are kept in a thermostat at a temperature and time,
optimal for growth of the used test microorganism:
for C. albicans - 27 ± 1 ° C and 7 days, after which the results are recorded
experiment.

AT quality control use test objects,
contaminated as indicated above and placed in water at the time
disinfection aging.

The multiplicity of the statement of the experiment must be sufficient for
obtaining statistically reliable results.

The regime (concentration-time-temperature) is considered to be effective;
Ensuring the death of the test microorganism on all test products
in the absence of its growth in a nutrient medium. Positive
A sample with a characteristic growth of a microorganism is
change in the nutrient medium (cloudiness, sediment, flakes, etc.) or
positive result (characteristic growth of a microorganism) with
re-dipping into a dense nutrient medium, or detecting

microorganisms in the solution of the neutralizer used. When
The presence of positive samples of the experiment is repeated, increasing
exposure time, but no more than up to 60 min.

If necessary, the effectiveness of the developed mode
is tested in practical conditions.

Criterion of decontamination efficiency - 100% death
C.albicans.

The disinfection time is not more than 60 min.

Note: the product must have a sterilizing
action.

1.3.3.3 Study of fungicidal effectiveness of DS,
intended for disinfection of dental impressions

In the development of regimes for disinfection of dental
C. albicans are used as a test microorganism. AT
The quality of the test objects is based on impressions from alginate,
silicone or other materials. For the production of impressions
The impression mass obtained in accordance with the recommendations
are placed in a plastic or metal spoon and
make an impression from plastic dentures with a modeled
gum. Impressions are applied to 0.1 ml of 1 mrdal suspension of C.albicans
(with the addition of 40% inactivated whey), dry them in
for 2-3 minutes, then completely immersed in a solution of DS. When
established fixing action of DS impressions before immersion
in a disinfecting solution is washed with running drinking water.
In parallel to control, contaminated impressions are immersed in
water. After a certain time (5-30 min.), The impressions are extracted from
solution and a gauze pad soaked with a neutralizer, make

washings. Napkins are placed in sterile test tubes with beads,
containing 10 ml of neutralizer, and shaken for 10 minutes.
Then, seeding the flushing fluid onto the nutrient media for
control the effectiveness of decontamination.

Criterion of decontamination efficiency - 100% death
C. albicans at the time of decontamination no more than 60 min.

1.3.3.4 Study of fungicidal effectiveness of DS, intended for disinfection of objects of care for patients, toys

When studying the effectiveness of DS, test objects are used,
imitating care items, or directly
items of care (liners, rubber heaters, ships,
objects from glass and plastic: thermometers, plastic
tips for enemas, etc.), toys (plastic, metal,
wooden, rubber, other than soft).

C. albicans are used as test microorganisms and
T.gypseum. For imitation pollution is used 40%

inactivated horse serum or large serum
of cattle. For this, before contamination of objects to the suspension
test-fungi add the necessary amount of serum.

Before contamination with test mushrooms, test objects are subjected to
mechanical cleaning - wash with water and soap and a brush.

After drying contaminated test objects
are placed horizontally and a test-
fungi at the rate of 0.5 ml of 2-billion suspension per 100 cm 2 area .
The suspension is uniformly distributed over the surface of the test objects
with a glass spatula, dried (until completely dry)

at room temperature 18-20 ° C and relative humidity
air 50-60%, then treated with a disinfectant solution.

The treatment of nursing and toys is carried out
methods of wiping, dipping and for large toys - irrigation
(drip).

The rate of disinfectant solution consumption during disinfection
methods of wiping or irrigation are determined depending on the
Method of processing is similar to experiments on disinfection
surfaces (see 1.3.3.6.). Double wiping or irrigation
spend 5-15 minutes. after the first.

When treated by immersion in a disinfectant solution
items for nursing and small toys the latter should
completely and with an excess of covering all objects. When immersed
small toys must be prevented from ascending.

The time for disinfection of objects is determined in the interval
from 15 to 120 minutes. depending on the type of test fungus and the availability of
organic pollution.

Control test objects are treated with sterile drinking
water from the same calculation as the experimental ones.

Control effectiveness of disinfection test objects
is carried out in the following way: gauze napkin (size 5x5 cm),
moistened in a solution of the neutralizer corresponding to the given

DS, thoroughly wipe the test object and immerse it in 10 ml of the same neutralizer, located in test tubes with beads. Waste time

gauze wipes 10 min. with constant shaking. Washable

liquid is sown on 2-3 cups of 0.2-0.5 ml per each on solid

nutrient media.

The crops are placed in a thermostat at temperatures of 27 ± 1 ° C and take into account the results after 2-28 days, depending on the type

test-microorganism.

The criterion of decontamination efficiency is not less than 100%

death of test fungi.

The time of disinfection of objects contaminated with C. albicans,

T.gypseum - not more than 120 min.

1.3.3.5 Study of fungicidal effectiveness of DS, intended for disinfection of linen

Effectiveness of disinfection of DC linen is determined with

using test objects, which are pieces of fabric from a calico

2 x 2 cm. The test cultures are C.albicans fungi

and T.gypseum. Sterile test objects impregnate 2 billion.

suspension of test fungi from the calculation of 20 ml for 10 test objects and

dried in a thermostat. Further, the contaminated test objects

placed in sterile coarse calico sacs the size of 5x8 cm for 2 pieces

in each.

When developing the disinfection regime for contaminated

suspension of fungi before contamination of test objects add 40%

inactivated serum (6 ml of 2-billion C. albicans suspension or

T.gypseum is mixed with 4 ml of inactivated serum) or 40%

fecal emulsion (6 ml of 2-billion C. albicans suspended with 4 ml

fecal emulsion). For the preparation of a fecal emulsion, 8 g

The faeces are ground in a mortar with 20 ml of water. Contaminated

test objects are dried in a thermostat at 37 ± 1 ° C for

20-25 min. or 1.5-2 hours at room temperature until complete

drying.

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When developing the disinfection regimes for clothes that are not contaminated secretions, the laundry is immersed in a container with a disinfectant solution at the rate of 4 liters of solution per 1 kg of dry laundry. When developing modes of disinfection of laundry contaminated with secretions, its immersed in a container with a disinfectant solution at a rate of 5 liters solution per 1 kg of dry laundry. Linen is immersed in a solution one after the other, making sure that things did not form air cavities, preventing process of disinfection. Pouches with contaminated test-objects are distributed between the layers of laundry (top, middle, at the bottom). After a predetermined exposure time of the disinfectant (eg 15, 30, 60 min.) Remove 1 bag from each level, sterile The test objects are taken out by tweezers, they are transferred to a solution neutralizer for 5 minutes, then washed for 5 minutes. in a sterile drinking water, then placed in liquid nutrient media. AT Control experiments instead of a disinfectant solution are used sterile drinking water.

The criterion of effectiveness is 100% death of test fungi.

Disinfection time (min.) Of laundry without visible impurities, Contaminated C.albicans, T.gypseum - no more than 240.

Disinfection time (min.) Of laundry contaminated with secretions and contaminated C.albicans, T.gypseum - no more than 240.

1.3.3.6 Study of fungicidal efficacy of DS, intended for disinfection of surfaces

Studies are carried out depending on the type of surfaces, their position (horizontal, vertical), method and multiplicity processing.

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When developing regimes of disinfection of surfaces use surfaces of 10x10 cm in size from various materials: smooth, rough, absorbent and non-absorbent (wooden, plastered, surfaces painted with oil, silicate, water-emulsion or glue paint; covered with wallpaper, surface from linoleum coverings, surfaces from painted or unpainted metal - stainless chromium-nickel acid-resistant steel, plastic, glass, artificial or natural leather, surface from tile - tile and metlakh, faience, etc.), but not less than 5 types of surfaces. As a test-cultures use C.albicans, T.gypseum; A.niger is used for development of disinfection of surfaces with a view to prevention and control of mold. To simulate pollution surfaces of sanitary equipment in case of contamination C.albicans, T.gypseum use protein contamination in the form of 40% inactivated horse serum; To simulate pollution surfaces of toilet bowls when C. albicans are contaminated contamination in the form of 40% fecal emulsion. For this, before contamination of objects to a suspension of fungi C.albicans, T.gypseum add the necessary amount of serum and to a suspension of C. albicans - the necessary amount of fecal emulsion.

Before contamination with the culture of fungi, the surfaces are exposed Mechanical cleaning - wash with water and soap and a brush (with the exception of surfaces covered with wallpaper and painted glue paint). The latter are wiped several times with a sterile a napkin moistened with sterile drinking water.

The dried surfaces are placed horizontally and on them A suspension of test fungi is applied with a pipette at a rate of 0.5 ml 2 billion

microbial suspension on an area of 100 cm^2 . Culture evenly spread over the surface with a glass spatula. Surfaces dried (until completely dry) at room temperature 18-20 ° C and relative humidity 50-60%, then treated with a disinfectant solution.

When studying the effectiveness of decontamination linoleum, tile

Metlakh, artificial or natural skin, glass
are placed horizontally, and the tree, painted with oil,
silicate, water-based or glue paints, surfaces,
wallpapered with wallpaper, plastic, tile and faience tiles -
vertically.

Surface treatment is carried out by rubbing or
irrigation (large-drop and aerosol).

To determine the rate of consumption for a single treatment
The disinfectant solution is applied by pipette to the surface
size 10x10 cm when applying the rubbing method in an amount of
1.0, 1.5 or 2.0 ml, and with large droplet irrigation, it is applied with
using a dispenser of 1.5-3.0 ml. In the aerosol treatment method
study the effectiveness of decontamination at the rate of consumption
30-50-100 ml / m^2 (depending on the type of aerosol generator and
used aerosol nozzle). Repeated wiping or
irrigation is carried out with an interval between treatments of 5-15-30 minutes.

The disinfection time of surfaces is determined in the interval
from 5 to 120 minutes. The choice of exposure depends on the purpose and
recommended conditions of use.

Control surfaces are treated with a sterile drinking
water also from the same calculation as the experimental ones.

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The tests are carried out at room temperature. When
need assess the efficiency disinfection
surfaces at a temperature lowered to minus 30 ° C
(the studies are carried out in a refrigerating chamber).

To control the effectiveness of disinfection of test surfaces
gauze napkin (5x5 cm), moistened in a solution
appropriate for this disinfectant
neutralizer, thoroughly wipe the test surface, then
immersed in 10 ml of the same neutralizer, located in test tubes
with beads. Wash time for gauze pad 10 minutes at constant
shaking. The washing liquid is sown (for 2-3 cups of 0.1-0.2 ml in

each) on solid differential-diagnostic nutrients
environment.

The crops are grown in a thermostat at a temperature of plus 27 ± 1 ° C.
The results are recorded for 2-7 days (C.albicans, A.niger)
and 21-28 days (T.gypseum) by counting the number of grown
colonies, then a density of contamination of 100 cm 2
surface and% disinfection, taking the number of colonies,
taken from the control surfaces, for 100%.

The criterion of the effectiveness of disinfection of surfaces -
not less than 99.99% of death of test fungi; Decontamination time (min.) at
contamination C.albicans, T.gypseum - no more than 240, A. niger - no more than
360.

1.3.3.7 Study of fungicidal effectiveness of DS,
intended for disinfecting dishes

Depending on the purpose of the DS,
disinfection of tableware and kitchen, laboratory and from
excretions.

AT quality test objects at development of regimes
disinfection of the dining room and kitchen utensils use plates,
glasses, mugs of various materials (porcelain, faience, aluminum,
glass, plastic, dishes covered with enamel); cutlery - knives,
forks, spoons from various materials (stainless steel,
aluminum, plastic); laboratory utensils (subject and coverslips
glasses, pipettes, Petri dishes, plates for immunological
analysis, etc.); dishes from under the secretions - bedboards,
urinals, pots, spittoons, etc. or their test objects
imitating. As test microorganisms for contamination
tableware use C.albicans, laboratory - C.albicans,
T.gypseum.

When developing the disinfection regimens for dishes with residues
food suspension of C.albicans mixed with oatmeal, semolina or other
porridge, cooked on milk with butter (to 10 g porridge
add 1 ml of a 2 billionth microbial suspension).

To simulate the contamination of tea ware use jelly
(10 ml of kissel is added with 1 ml of a 2-billion suspension of C. albicans)
laboratory glassware - 40% inactivated whey
(6 ml of a 2-ml suspension of C.albicans or T.gypseum is mixed with 4 ml
inactivated whey), and dishes from under the secretions - 40%
fecal emulsion, contaminated with C.albicans (10 ml - 1 ml
$2 \cdot 10^9$ suspension).

Before contamination with test mushrooms, dishes (table and
laboratory) and cutlery are subjected to mechanical cleaning
- Wash with soap and water. The dishes are placed horizontally and
a suspension of test fungi is applied to it with a pipette at a rate of 0.5 ml of 2
billion suspension per area of 100 cm^2 . Culture evenly

spread on the surface of the dishes with a glass spatula. Canteens
The contamination devices are immersed in a bacterial suspension for
1-2 minutes, leaving their pens uninfected.

The dishes are dried (until completely dry) at room temperature
temperature 18-20 ° C and relative humidity 50-60%, then
treated with disinfectant solution

Processing of a dining room, tea, laboratory utensils and dining rooms
devices are carried out by immersion in a disinfectant solution.
The solutions are prepared in drinking water. The temperature of the test solution
18-20 ° C. If necessary, study the effectiveness of solutions,
having a temperature of 50 ° C.

Control is similarly contaminated dishes,
which is immersed in the same volume of sterile drinking water.

The disinfectant solution must be completely and excessively
cover all the dishes (table and laboratory) and cutlery
(at the rate of not less than 2 liters per 1 set).

After certain time intervals (for example, 15, 30, 60 min.
etc.) are extracted from the disinfectant solution in one subject
(for example, a plate, glass, slide, knife, etc.) and sterile
gauze cloth (size 5x5 cm), moistened in a solution
a neutralizer corresponding to this DS; carefully wipe
the contaminated part of each item, immerse the napkin

in 10 ml of the same neutralizer, located in test tubes with beads.
Wash time for gauze napkins 10 min. at constant
shaking. After washing, the gauze pad is immersed in
the corresponding liquid nutrient medium. Washing liquid
sow 2-3 cups of 0.2-0.5 ml per each on a solid nutrient
environment.

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The crops are placed in a thermostat at a temperature of 27 ± 1 ° C and take into account the result after 2-7 days (C.albicans) and 21-28 days (T.gypseum).

The disinfection time of the dishes is determined in the interval from 15 to 240 minutes. depending on the type of test microorganism and the availability of pollution.

The criterion of decontamination efficiency is not less than 100% death of test fungi.

The time of disinfection of tableware without food leftovers, Contaminated C.albicans - not more than 60 min.

Time disinfection utensils from residues food, Contaminated C.albicans - not more than 120 min.

Time disinfection laboratory utensils, Contaminated C.albicans, T.gypseum - not more than 120 min.

Time disinfection utensils from under excretions, Contaminated C.albicans - not more than 120 min.

1.3.3.8 Study of fungicidal effectiveness of DS, intended for decontamination of secretions

The DS intended for the disinfection of possess the ability to homogenize an organic substrate (feces, sputum). Preparations that do not have this property for disinfection of discharge is not suitable.

The study of the activity of disinfectants during processing separation are carried out taking into account their consistency and the relationship with disinfecting solution or dry preparation.

As test microorganisms in the development of regimes

disinfection of secretions using C.albicans.

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Determination of the effectiveness of urine disinfection is carried out as follows: take several tubes, pour in them for 9 ml of urine, add 1 ml of a suspension of C.albicans containing $1 \cdot 10^9$ μ / ml. Undiluted DS or its solutions are added to the urine in different ratios (equal, double, etc.). At the end of of the exposure time (15, 30, 60, 90, 120 min.), pipet 1 ml of the test mixture and transferred to a neutralizer of 9 ml, and then from 1 ml of the mixture in a tube with 5 ml of broth. After careful mixing 1 ml is transferred to a second tube with broth, and then make crops of 0.1 ml on solid nutrient media, both from the first, and from the second test tubes. Petri dishes with crops are placed in a thermostat.

Control is similarly performed experiments with adding to the urine is not a disinfectant solution, but a sterile drinking water.

An effective DS is considered effective, providing 100% death C.albicans in 3 experiments with concurrent results.

Definition effectiveness of disinfection faeces: 20 g of fecal matter is ground in a mortar and 80 ml of sterile water are added. The resulting emulsion is filtered through a double layer of gauze, is poured into tubes of 9 ml and 1 ml of a suspension of culture C. albicans containing 1×10^9 μ / ml.

The prepared emulsion of feces is filled with equal or with a double amount of disinfectant solution or a different amount of a dry preparation. After contact with DS produce seedings in the same way as in the disinfection of urine. The results are taken into account after two days.

With positive results, experiments with large number of decorated feces (200-250 g). To do this, place them

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in a vessel and pour a disinfectant solution in an equal or
double the amount with respect to the weight of feces or fall asleep dry
drug. Then a small part of the fecal masses of the glass
The stick is mixed with the liquid, and the remaining mass is left in
the form of small lumps. At regular intervals
(30, 60, 90 and 120 minutes) separate the liquid part and
lumps.

The liquid part of the fecal masses is pipetted and produced
sowing the same way as urine. Dense parts of feces are taken up by a loop and
is lowered into 5 ml of nutrient medium by ripping them on the edge of the tube and
thoroughly mixed with broth; then transferred from this tube
1 ml of the mixture into a second tube, also containing 5 ml of broth. how
From the first, and from the second test tube, 0.1 ml of
Petri dishes with a dense nutrient medium.

Control is similarly performed experiments with
adding sterile water to the fecal emulsion instead of DS.

The effectiveness of the examined DS is judged on the basis of 3 experiments
with matching results. Effective means,
providing 100% death of C.albicans in decontaminated
material.

The criterion for the effectiveness of disinfection of the precipitates is 100%
doom test-fungus. Time disinfection excretions,
Contaminated C.albicans - no more than 6 hours.

1.4 Methods for determining the activity of antimicrobial materials
(fabrics, paint and varnish coatings).

Determination of the activity of antimicrobial materials is carried out
complex using the methods: "agar plates" and
Drip application of test microorganisms to test samples from

antimicrobial materials.

Evaluation of antimicrobial tissue activity. Definition antimicrobial activity of textile materials is carried out with using the "agar plates" method. When evaluating activity tissues are controlled by tissue samples of the same articles, not containing antimicrobial substances.

To determine the antimicrobial activity of tissues in dependence the following test microorganisms are used for the intended purpose: S. aureus, E. coli, Mycobacterium terrae, T.gypseum.

The "agar plates" method gives a qualitative estimate antimicrobial activity of the test samples, as it allows determine the presence or absence of an antimicrobial effect.

Technically, it can be performed in two ways.

Method No. 1. Melt in a water bath and chilled up to 45 ° C nutrient agar (100 ml) is mixed with 1 ml of a suspension of test-microorganism containing 10^8 cfu / ml and poured into petri dishes for 20 ml. On the surface of the frozen agar, test samples are applied of the test tissue 2 x 2 cm in size. The plates are placed in a thermostat at 28 ° C or 37 ° C, depending on the test microorganism. Accounting of antimicrobial activity against S. aureus and E. coli spend 24-48 hours, with respect to Mycobacterium terrae - through 5 days, in respect of T.gypseum - after 21-28 days, determining the size of the zones of growth retardation of microorganisms by measuring

distance from the edge of the test sample to the growth boundary of microorganisms around the dough.

Method No. 2. To a 16-hour broth culture of E. coli or S. aureus add 0.9% solution of common salt in a ratio of 1: 2. AT sterile Petri dishes are poured into 12 ml of nutrient agar; after congealing on its surface is evenly distributed with a spatula 0.1 ml of a prepared suspension of test cultures; then from above place test samples of a tissue 2x2 in size. Cultivation of test cultures and registration the results are carried out as in the first method.

Criteria for laboratory effectiveness evaluation

antimicrobial tissues.

Antimicrobial tissues studied by the method of "agar plates "are considered effective if the growth retardation zone of test-microorganisms not less than 4 mm.

Antimicrobial activity of tissues should not decrease with multiple (not less than 10) washes more than 15%.

Evaluation of the antimicrobial activity of paint and varnish coatings.

Definition antimicrobial activity paint and varnish coatings are carried out using drip application test microorganisms. As test microorganisms are used E. coli and S. aureus. Depending on the intended area application of antimicrobial coatings a list of test microorganisms can be expanded (Mycobacterium terrae, C. albicans, A. niger).

On a test surface measuring 10 × 10 cm, colored antimicrobial paint or coated with antimicrobial varnish, 0.5 ml of the suspension of the test microorganism is applied by a sterile pipette, containing $2 \cdot 10^8$ CFU / ml. After the necessary disinfection exposure for a certain period of time during the period

from 30 minutes to 24 hours from the surface take a wash with sterile gauze a cloth moistened with a sterile neutralizer solution. Napkins are placed in test tubes with beads containing 10 ml neutralizer and within 5-10 minutes. shake. Then conduct sowing flushing fluid on dense nutrient media. Crops are kept in a thermostat at a temperature and time, optimal for growth of the used test microorganism. As a control use surfaces painted with paint or varnished, not containing antimicrobial substances. Activity of paint and varnish materials are evaluated by calculating the percentage reduction in microbial seeding of the prototype as compared to the control sample.

The criterion of antimicrobial activity of paint coatings - Decrease of seeding not less than 90% after 24 hours after application of the test microorganisms mentioned above.

Study prolonged actions paint and varnish materials. Study of prolonged action of paint and varnish

coatings are carried out on test surfaces with additional artificial contamination with test microorganisms.

On the test surface, regularly once a week for 6 months 0.5 ml of a suspension of test microorganisms containing 10^6 cfu / ml. The effectiveness of disinfection is estimated 1-2 times per month.

The criterion of antimicrobial activity of LMC - decrease at least 90% after 24 hours after application test microorganisms and prolonged antimicrobial action less than 6 months.

1.5 Methods for determining the efficiency of DS in practical conditions

1.5.1 General

A study of the effectiveness of DS in practical conditions is The final stage of the study, according to which results are given recommendations for its industrial production and use in practice of medical disinfection. Practical trials in cases where the DS contains a new active substance and require confirmation of the effectiveness of the developed modes.

The purpose of practical tests is to clarify the target purpose, conditions of use, an assessment of the effectiveness and safety of the developed modes of disinfection, reliability recommended precautionary measures, influence on the invoice and functional properties of processed objects, detection of advantages and disadvantages in comparison with the analogues used.

Tests are conducted in medical organizations and / or infectious foci in accordance with the Program and Methodological instructions for testing the DS, which are compiled on the basis of results of earlier studies on physicochemical properties, antimicrobial and disinfectant activity, and

toxicity in the laboratory.

The Program should specify the time (duration), place carrying out and the purpose of tests, the list of objects of processing, control parameters, volume (number of washings).

Methodological instructions for conducting tests should contain specific information about the appointment of the DS, its characteristics (description, physico-chemical and antimicrobial properties,

toxicity), methods of preparation of working solutions, conditions and regimes of application, rules for safety and first aid for poisoning.

Practical tests of DS are carried out by employees treatment or prophylactic institution or state establishments of disinfection profile, or Centers for Hygiene and epidemiology, or state unitary enterprises, or disinfection station. Current disinfection is carried out in one or two multidisciplinary medical organizations, and the final - no less than 10 infectious foci.

Before carrying out the tests, an entrance chemical control for compliance with the pilot batch of DS requirements Technical conditions, and instruction of medical personnel, conducting the tests.

1.5.2 Determination of the effectiveness of DS in the decontamination of various objects (surfaces, linen, dishes, etc.)

Evaluation of the effectiveness of the DS is carried out on the basis of detection sanitary-indicative and pathogenic microorganisms in flushes, taken from the objects before and after disinfection.

When testing DS in the foci of bacterial infections, control efficacy is performed to detect E. coli - in intestinal infections; S.aureus - with infections of the respiratory tract; S.aureus and M. tuberculosis - with tuberculosis (M. tuberculosis is defined only in foci with abundant bacilli in patients or in assessing sputum disinfection efficiency). With viral and fungal

infection, disinfection quality control is carried out on the basis of

detection of E.coli

S. aureus.

In MO, the effectiveness of a disinfectant is judged by
Detection of the sanitary-indicative microflora (E.coli and S.aureus),
as well as causative agents of nosocomial infections (P. aeruginosa,
P.vulgaris, C.albicans, A.niger and others), depending on the profile
hospital and the presence of nosocomial infection.

To assess the effectiveness of DS in practical conditions, they do not take
less than 50 washings (25 before and after disinfection). Samples after
disinfection is selected immediately after the end of the exposure and delivered
for analysis in the laboratory no later than 2 hours from the moment of their selection.
Samples are selected from sites of epidemiological significance,
the possibility of re-contamination of which is excluded
specificity of their use.

With limited practical tests conducted with
participation of DS researchers, because of the complexity of isolation and
cultivation of certain pathogens is allowed
test objects, artificially contaminated with microorganisms in
laboratory (for example, in the disinfection of laundry with tuberculosis,
use tests, seeded Mycobacterium terrae, and with fungal
diseases - T.gypsem).

Accounting for the effectiveness of decontamination is carried out by qualitative and
quantitative methods.

With a qualitative method for assessing the effectiveness of the samples taken with
objects before and after disinfection, are sown on the differential-
Diagnostic environments and account for the presence of a specific
growth microorganisms, identifying percent detection
microorganisms before and after disinfection. In the quantitative method

Selected samples are inoculated on solid nutrient media,
count the number of CFUs and determine not only the percentage
positive samples, but also the contamination density of a unit
surface.

After disinfection of surfaces in rooms, furniture,
sanitary equipment, utensils, toy samples
are selected by wiping the surfaces of the listed objects
an area of 100 cm 2 moistened in neutralizer solution
Sterile gauze napkins measuring 5x5 cm or cotton
tampons.

With a qualitative method for assessing the effectiveness of the samples taken with objects before and after disinfection, are sown on the differential-
Diagnostic environments and account for the presence of a specific
growth microorganisms, identifying percent detection
microorganisms before and after disinfection. In the quantitative method
Selected samples are inoculated on solid nutrient media,
count the number of CFUs and determine not only the percentage
positive samples, but also the contamination density of a unit
surface.

When controlling the effectiveness of disinfection of small items
flushing is taken from the entire surface (or completely immersed in the
nutrient medium), and objects of a large area - with 2-3 sites,
with a total area of 100 cm 2 .

The effectiveness of the means for disinfecting objects in
practical conditions are assessed by comparing the contamination of objects
before and after treatment.

The efficiency criterion for the final
disinfection - sowing microorganisms no more than 0.5%, with the current

disinfection at home - no more than 3%, with the current disinfection in
treatment and prevention institutions - sowing of non-pathogenic
microflora no more than 2% of selected flushes.

1.5.3 Determination of the effectiveness of DS in disinfection medical devices

Medical products after use in the patient are divided on 2 groups. From the products of the 1st group take the wash to determine microbial background during testing. Medical products (including endoscopes) from the 2nd group are disinfected in accordance with regimes and under the conditions recommended in the Guidelines (Instructions) for testing a particular facility.

Quality control of disinfection of medical devices is carried out by the wash method. Taking washings produce sterile gauze napkins size 5x5 cm, sterilized in paper bags or petri dishes.

Control is subject to 1% of the simultaneously processed products one name, but not less than 3-5 units.

Before taking washings from objects to wide-necked test tubes with Glass beads are dispensed by a sterile pipette in 10 ml sterile neutralizer corresponding to the disinfectant. Wetted with a sterile cloth wipe the surface of the product, after which the napkin is placed in test tube with neutralizer solution and 5 min. shake.

In articles having channels, the working end of the article is lowered in test tube with a sterile neutralizer and with a sterile syringe or pipette 1-2 times wash the channel with this solution. After

this from the flushing liquid is produced by sowing on the differential-diagnostic environments.

The disinfection of endoscopes is controlled by the method of taking washings from endoscope sites that are difficult to access and disinfection, for example, the distal end of the endoscope, and by microbiological control of the flushing liquid, first of all, from the instrumental channel of the endoscope, as well as other channels, cavities and surfaces.

The quality of disinfection after it is judged by the absence

on medical products of Staphylococcus aureus, Pseudomonas aeruginosa sticks and bacteria of the colibacillus group.

To detect microorganisms in the flushing liquid are passed through a membrane filter and then placed on a surface of a dense differential-diagnostic environment. When the absence of a filtering device flushing liquid of 0.1 ml are sowed on the surface of the yolk-salt, blood agar and medium Endo. The crops are kept in a thermostat at plus 37 ° C for 48 hours.

In the presence of growth of microorganisms on agar their identification carry out in accordance with the current methodological documents.

1.5.4 Determination of the activity of products from antimicrobial tissues

Evaluation of the effectiveness of products from antimicrobial tissues conducted in medical organizations and on industrial enterprises, where working conditions contribute to the development of diseases the skin of microbial etiology, or on which provide a small amount of microorganisms in the air and on surfaces.

A set of products from antimicrobial tissue to be tested, specific for each institution. Usually in the MO and on enterprises are underwear and bed linen, overalls of personnel.

In all institutions, along with products from the test tissue (experimental group), a group of similar products from the conventional tissue (control group), preferably the same article as experienced group.

Microbial contamination of products from antimicrobial tissues study by fingerprint method. Cut glass, cut in half, spread out with sterile tweezers in sterile cups Petri (three halves of a slide). The nutrient medium (depending on the test microorganism - casein or Meat-Peptone Agar, Saburo Agar, Potato-Glycerin Agar) melt in a water bath and using a pipette, observing rules aseptic, poured on the surface of each glass before it

of the complete coating (1.5-2.0 ml). Cups with plates after hardening
The medium can be stored for up to 3 days in the refrigerator.

The capture of fingerprints is carried out as follows:
having opened a cup with plates, take a sterile tweezers
Plate by the edges, without touching the nutrient medium. Remove the plate
from a cup and tightly applied to the test medium
surface for 2-3 seconds. After contact with the surface
A plate with a nutrient medium prints upward into a cup
Petri. The cup is closed with a lid and placed in a thermostat at 37 ± 1 ° C
for 48 hours, after which the count of the grown up colonies is calculated. AT
the natural location of microbial
cells.

In determining the contamination of laundry samples are selected by
prints from the inner surface of the underwear in the area
The back (the upper edge of the scapula), the midline of the abdomen between the navel and
Breast, breast / thoracic region, above the breast nipples. Samples
They are selected symmetrically from the left and right sides. With outdoor
The surface of the undergarment is taken in the axillary region and in
area of the shoulder seam.

From the pillow cases, towels are sampled at three points. FROM
duvet covers, sheets, soft inventory / curtains, screens, bags
for laundry / take 3-5 samples at the corners and in the middle.

Antimicrobial activity of tissues should not decrease with
multiple (not less than 30) washes more than 15%.

The efficiency criterion is the absence of growth of microorganisms in
selected samples.

1.5.5 Determination of the antimicrobial activity of paint and varnish Coatings

Definition antimicrobial activity paint and varnish
materials in practical conditions is the final stage
The study, which results in recommendations for

application in the practice of medical disinfection.

In accordance with the purpose of paintwork materials

The surfaces in the room are painted with paint or varnished.

Control surfaces of other similar premises serve,

treated with paintwork materials that do not contain

bioadditives.

Assessment of the antimicrobial activity of paint and varnish coatings

conducted on the basis of the detection of sanitary and

pathogenic microorganisms in washings taken from surfaces,

painted with test paint or varnished. As

Surfaces that are painted or coated

varnish that do not contain bioadditives.

Evaluation of the antimicrobial activity of paint and varnish materials

quantitative method: selected samples are sown on

solid nutrient media, count the number of CFUs and determine

percentage of decrease in microbial contamination compared to

control.

Antimicrobial coating is considered effective in providing

Reduced seed contamination compared with control

Surfaces after 24 hours not less than 90% and duration

antimicrobic effect for at least 6 months.

1.6 Methods of studying and evaluating virucidal activity disinfectants

1.6.1. General Provisions

Methods for studying and evaluating the virucidal activity of DS

harmonized with the European standard 14476. "Chemical

disinfectants and antiseptics. Virucidal quantitative

Suspension test for chemical disinfectants and antiseptics,

Used in medicine - Test methods and requirements (phase 2 / step

1), 2005; "The protocol for testing the effectiveness of disinfectants,

used to inactivate the hepatitis B virus of ducks and maintain

corresponding level of requirements ", EPA 2000, USA; "Commissions

on anti-virus disinfection of the German Association for Combating virus diseases (DVV) ", 2004.

Viruses have different resistance to physical and chemical factors, including DS and substances. Sustainability viruses to DS is determined by their structure and chemical composition. More resistant to the action of DS viruses without a lipid-containing shells, for example, picornaviruses, parvoviruses. Viruses with lipid-containing envelope, for example, influenza, herpes, HIV relatively easily inactivated. The mechanism of action of DS for viruses is different and depends on the chemical composition. They can selectively damage to the nucleic acid of the virus, nucleocapsid proteins or lipoproteins of the supercapsid shell. Perhaps synchronous influence on these structural elements, disintegration of the viral particles or blocking of receptors on its surface due to accumulation of amphoteric disinfectant substances. For rate virucidal activity, several test viruses should be used with different stability, which makes it possible to evaluate the activity of DS in against a wide range of viruses.

The results of studies of virucidal activity of DS depend on also on the nature of the virus-containing material used, method of indicating the virus, a method for determining the virucidal properties DS, the composition of the tested DS (active substances and other Components).

When studying the virucidal activity of DS, it is necessary to have its detailed description with indication of the formulation, physico-chemical properties (including solubility and stability), confirmed by the results of chemical input control.

The DSs accepted for research should meet the following requirements: to have good solubility in water, to maintain its activity in the presence of organic substances (blood, etc.)

biological substrates), be non-toxic or slightly toxic for people (and animals), not to have an unpleasant smell, not to spoil disinfected items.

An indispensable condition for the study of virucidal activity of the DS is the use of a neutralizer.

1.6.2. Test viruses, model systems, evaluation criteria and basic conditions that must be observed in research virulicidal activity of DS and their substances. Application of the neutralizer

Test viruses. Model systems for DS and substances. In studies of the virucidal activity of DS it is necessary to use both RNA- and DNA-containing test-viruses (Appendix 1). In this case, the presence of laboratory model, safety for people working with the virus.

It is mandatory for all DSs to test two test-viruses: a type 1 poliomyelitis virus (the Sabin vaccine strain (LSc-2ab) (hereinafter "poliovirus")) and adenovirus type 5 (strain Adenoid 75) (hereinafter referred to as "adenovirus")).

A remedy that has shown the ability to inactivate polio adenovirus, are included in the DS group with virucidal activity. DS with virucidal activity can be used for disinfection for any viral (including especially dangerous) infection that has value in human infectious pathology.

For DS, used at elevated (up to plus 60 ° C) temperature solutions (for example, for disinfection of laundry), As a test virus, bovine parvovirus (Haden strain) is used.

In connection with the epidemiological and social significance hepatitis A, for disinfection in infectious foci also carry out Studies with the hepatitis A virus. To study and evaluate activity

DS with respect to the hepatitis A virus uses the hepatitis A virus human (strain HAS-15). DS, inactivating the hepatitis A virus, is included in the group of agents with virucidal activity.

To study the virucidal activity of DS with respect to the virus hepatitis B use surrogate virus - hepatitis B virus ducks (HLPV), with respect to the hepatitis C virus - a surrogate virus - a virus diarrhea - diseases of mucous membranes of cattle VD-BS (strain VK-1B1-No. 28) or human hepatitis C virus (strain D1).

The spectrum of test viruses can be expanded, in this case give additional information on the results of specific virus.

Model systems for the investigation of DS and substances. In vitro studies in cell culture. In the experiments, cell culture, sensitive to the test virus.

In vivo studies. In the experiments, sensitive laboratory animals (white mice), ducks, etc. (Annex 1).

Criteria for evaluating the virucidal activity of DS and substances. The virucidal DS (substance) should suppress infectivity mandatory for testing test viruses - poliovirus and adenovirus on the investigated objects no less than 4 log10 TCDD50 (that is, the degree of inactivation should be at least 99.99%).

Criterion of virucidal activity of DS (substances) for other test-viruses (including viruses-agents of especially dangerous infections)

is the absence of infectiousness, determined by modern methods of indication.

The degree of inactivation of the test virus is determined in sensitive model systems for the suppression of infectious, cytopathic or plaque-forming activity of the virus in a cell culture, or absence of markers of infection, or by specific death laboratory animals (white mice), etc. For more The test-virus should be used with the maximum titre.

The indicator of virucidal activity of DS (substances) is
The rate of inactivation, which is the ratio
concentration of the test virus, expressed in decimal logarithms, before and
after exposure of the DS for a certain period of time
(exposure). Power reduce viral infectiousness
is calculated in decimal logarithms by the difference of virus titers before and
after processing DS.

The main conditions that must be observed in research
virulicidal activity of DS and substances:

investigated DS before, during and after studies (trials)
should be stored in accordance with the requirements of technical specifications
(TU). In the absence of technical specifications, it is necessary to comply with the general requirements:
Avoid exposure to direct sunlight and moisture, exposure
high temperatures and freezing;

When conducting certification tests, DSs are stored in
compliance with the rules of certification;

Dosage solutions should be prepared on a sterile tap
dechlorinated water;

To maintain the temperature of the solutions of the DS under study during
of the whole experiment within 200 ± 2 ° C (if by conditions
experiment, another temperature is not recommended), regardless of
ambient temperature;

Use working solutions of DS produced in the form of
granules, powders, tablets, etc. only after their complete dissolution;

the multiplicity of the experiments should be at least three
(provided that the same results are obtained);

In the absence of a cytopathic effect, the samples are subjected to
necessary processing for the purpose of the second, and with
necessity and the third blind passage for definition of completeness
inhibition of the test virus;

experiments should be accompanied by all necessary
control, including control of completeness of neutralization

disinfectant, viability of the test virus, contamination of test-
the object of the test virus, the culture of cells or laboratory animals
(mice), ducks, etc .;

use a neutralizer matched to a specific DS (or
disinfectant substance, further in this section - "DS");

When researching the DS, the recommended
measures of individual protection (gloves, glasses, respirators, etc.).

Application of the neutralizer of DS and substance. To neutralize
DS (substances) use a neutralizing agent (or a complex of
several substances), stopping the action of DS. Neutralizer
either added directly to the nutrient medium, or
wash them test objects after exposure to DS (substance,
substance) in order to stop its effect on
test-virus after a specified time (exposure).

To neutralize the DS in the form of a mono-drug based on
oxidizers (chlorine, iodine, oxygen-containing agents) are used
0,1-1,0% solutions of sodium thiosulfate; for halo-negative (chloro-,
bromine and iodate) and oxygen (hydrogen peroxide, its
complexes with salts, peracetic acid, ozone) - 0,1-1,0% solutions
sodium thiosulfate; for quaternary ammonium salts
(alkyldimethylbenzylammonium chloride, didecyldimethylammonium chloride
and etc.), derivatives guanidine (polyhexamethyleneguanidine
hydrochloride, chlorhexidine bigluconate, etc.) - 0,1-1,0% solutions
sodium lauryl sulfate (sulfonol) or solutions of lauryl sulfate
sodium with 10% skim milk or complex neutralizer
(see below); for aldehydes (glutaraldehyde, glyoxal,
formaldehyde, orthophthalic aldehyde) - 1.0% solution of pyrosulfite
(metabisulphite) sodium or a complex neutralizer (see below);
for acids - alkali in an equivalent amount; for alkalis -
acids in an equivalent amount; for alcohols - dilution in water
up to inactive concentration; for compositional means -
"Complex" neutralizer, for example, containing Tween 80 (3%),
saponin (0.3-3%), histidine (0.1%), cysteine (0.1%).

If the composition of DS contains oxidants, the neutralizer additionally, sodium thiosulfate is added. It should be kept in mind that complex neutralizer has a pronounced cytotoxic action on cell cultures.

If you can not find any of the listed neutralizers, which is manifested in nonspecific degeneration cell culture (or death of mice, ducks), then use 60% -80% Serum (without preservative) of cattle (SSRS), inactivated at 56 ° C for 30 minutes. Serum (without

preservative) of cattle neutralizes a large list of DV and is closest to the complex neutralizer.

If it is impossible to neutralize the toxic effect of DS additional methods of removal of DS-dialysis mixture virus-disinfectant, for example, using Sephadex LH-20, G-75; sedimentation virus method high-speed centrifugation or filtration through membrane filters.

1.6.3. The main stages and methods of studying the virucidal activity of DS and substances.

The study of DS virucidal activity is carried out in two phase:

1) the first stage of the research is to determine whether virucidal activity, is carried out in vitro by a suspension method or by the method of cambric test objects;

2) the second stage of the research is the study of the virucidal the effectiveness of the DS in the processing of various test objects, air contaminated with the test virus.

As test objects you should use different products medical purpose; nursing and toys; linen, overalls and other articles made of fabrics; dishes, including laboratory; various surfaces in the premises (hard furniture, various items, equipment, including sanitary and technical and etc.); blood; excretion (urine, feces, sputum).

The volume of research and the list of objects of disinfection

depends on the purpose of the DS, their composition, toxicology characteristics, forms of issuance of funds, etc.

1.6.3.1. Suspension method.

To a virus suspension (VS), which may be used culture fluid after removal of cellular residues, or sun organs or tissues, or blood serum, add the test substance in a volume of 1: 9 (1 volume of the virus and 9 volumes agents) in various concentrations.

Suspension test is carried out in two versions: without protein load and with a protein load. In the latter case, the virus Inactivated serum of bovine livestock at the rate of 40% of its concentration in a mixture of virus - disinfectant.

The resulting mixture (both with and without serum) is maintained at room temperature (20 ± 2 ° C) for 15-30-60 minutes, neutralized (in a ratio of 1: 1, i.e., 1 mixture volume and 1 volume neutralizer), shaking for 5-10 minutes, and used for test-virus in a sensitive cell culture (or on mice, or on ducks).

The procedure for determining an infectious virus after exposure DS (substance): the test material (mixture of virus, DS and neutralizer) is added (or contaminated with the test material mice or ducks) in wells plate (test tubes) with grown monolayer of cells or in a suspension of cells (in case of application suspension culture of cells), after 30-60 minutes. remove the mixture, replace it with a culture medium. The cell culture is incubated in thermostat at the temperature required for reproduction of the virus in during the observation period.

On virucidal activity, agents are judged by the presence or The absence of a cytopathogenic action caused by a virus, or other manifestations indicating the reproduction of the virus.

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All experiments are accompanied by cell culture controls,
virus, completeness of neutralization.

Effective means (substance) providing
inactivation of the virus at exposure time not more than 60 minutes.

1.6.3.2. Quantitative suspension method
(Republic of Belarus).

The temperature of all ingredients (test-virus suspension, 2%
solution of serum albumin, serum of large embryos
Cattle, bidistilled water and breeding of DS) are brought to
20 ° C. One part of the test virus suspension is mixed with one part
serum albumin solution, or one part serum
fruits a major horny cattle, or one part
bidistilled water. Then each of these groups is added
eight parts of diluted DS in a concentration of 1.25 times more
higher than the final (studied) concentration.

The efficacy of DS was tested in groups without protein and with 0.2%
serum albumin or with 10% serum of large embryos
Cattle (final concentrations are indicated). Control groups
The test virus should contain the same protein concentration.

In the event that the suspension method is performed in option c
neutralization of the action of the DS, after the end of the exposure period to
mixture of a suspension of test virus and solution of DS add an equal
amount of neutralizer solution for 5 min.

Then, successive dilutions of the mixture are prepared
cooled (0-40 ° C.) support medium (buffered saline
solution) in steps of 1:10 and the test tubes (vials) are immediately placed on
ice. These dilutions infect a sensitive culture

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cells, and according to the results of taking into account the signs of reproduction of the test virus
determine its residual infectivity.

Since the preparation of a series of dilutions occurs
multiple dilution of DS, it is possible to set up the method,
when the neutralizer is not used. In this case, add
control group of the beginning of the exposure, in which the preparation
successive dilution and infection in advance
prepared culture cells are produced immediately after
combining the suspension of the test virus and the solution of DS. Decrease
infectivity of the virus is determined in this case as the difference of titers in
groups of the beginning and end of the exposition. This way is possible
determine the real effectiveness of the DS, despite the possible
The continuing virucidal and virus-inhibiting effect.
Toxicity of DS for cell culture under such an approach is reduced in
successive dilutions and, as a rule, does not go beyond
dilutions 1: 1000. The initial titer of the test virus in this trial
should be sufficiently high, not less than 7-8 lg TITD 50 / ml
(PFU / ml). In this regard, this version of the method is more
is suitable for highly reproductive test viruses, in particular,
poliovirus and similar in terms of the degree of resistance of enteroviruses,
for example, the entero virus ECHO 6.

A prerequisite for carrying out the test is
use of a number of controls:

a) control of the virus: one part of the test-virus suspension is mixed
with 9 parts water. Such control is provided for each
exposure time or use the maximum exposure;

b) control of the virus with serum albumin or serum:
one part of the virus suspension is mixed with one part of the solution

serum albumin or one part of a large serum
cattle, then add 8 parts of water;

c) toxicity control of DS: two parts of buffered
isotonic sodium chloride solution (PBS, Dulbecco modification),
mixed with 8 parts of diluted DS. At the same time with
definition of infectiousness, prepared serial dilutions
this mixture is applied to a cell culture. If the DS cytotoxicity is so
it is high that a decrease in the infectious titer of the test virus by 4 log10

TCD50 / ml (PFU / ml) in comparison with the control of the virus can not be achieved, use a suitable method of reducing it;

d) control of the toxicity of the mixture DS + neutralizer: two parts of PBS are mixed with 8 parts of diluted DS and combined with an equal volume of neutralizer solution. Simultaneously with the definition infectiousness, prepared serial dilutions of this mixture is applied to the cell culture. Control is used in case of fulfillment suspension method in the variant with neutralization of the DS action;

e) control of completeness of neutralization of DS (or virucidal actions of neutralization products and neutralizer residue): one part of PBS is mixed with 8 parts of diluted DS and combined with 10 parts of neutralizer solution. After 5 minutes. add one part of the test virus slurry. Simultaneously with the definition infectiousness, prepared serial dilutions of this mixture is applied to the cell culture. Control is used in case of fulfillment suspension method in the variant with neutralization of the DS action;

f) control of the beginning of exposure: all procedures are performed in order difference, that dilutions of a mixture of a suspension of test virus and DS are prepared in the time when the exposure time starts for other samples. Difference between the titers of the virus in this control and in samples with exposure

helps to determine the extent of the infectivity of the virus only for Exposure time (the possible continued action is subtracted DS in the course of breeding and determining the infectivity of the samples). Control are used in the case of a slurry method in the variant without neutralization of the DS action;

g) control disinfectant actions (reference-disinfectant): one part of the test virus suspension is mixed with 4 parts of phosphate buffer (0.1 M, pH 7.0) and 5 parts 1.4% solution of formaldehyde. Exposure - 5, 15, 30 and 60 minutes (for poliovirus, enterovirus ECHO 6 - 15, 30, 60 and 120 min.).

The virucidal activity of DS is calculated as the difference between infectious titer of the test virus in the control of the completeness of neutralization DS and infectious titre determined after interaction

virus and DS in the main groups. If the research is carried out in variant without the use of a neutralizer, virucidal activity is calculated as the difference between the virus titer in the start control exposure and its title after the completion of the exposition.

It is believed that the DS solution has a sufficient virucidal activity, if within the recommended exposure Infectious titre of the virus is reduced, at least, by 104 (4 log10) TCD50 / ml (PFU / ml).

1.6.3.3. The method of cambric test objects

Studies of the virucidal activity of DS (substances) by this method is carried out on test viruses, fixed on batistovyh test-objects. When using this method, some loss (1-2 log TCD50 / ml) of viral particles during the immersion contaminated test objects into the solution of DS, neutralizer and

subsequent laundering from residues of DS. However, this method is more It is acceptable for the study of DS whose neutralization products are toxic for cell culture and cause in them nonspecific degenerative changes.

Preparation of the experiment.

The viral suspension is contaminated with batistovye test objects (hereinafter referred to as "tests").

As tests, batches of 1 x 0.5 cm, pre-stretched (i.e., liberated from starch) and ironed. The tests are placed in a sterile Petri dish and is filled with a virus suspension at a rate of 0.05-0.1 ml of liquid per one test (without serum or with the addition of 40% serum of a large cattle). The tests are dried at room temperature not less than 60 min. Contaminated with viral suspension tests used in experiments. Tests should not be harvested for future use, their are contaminated with a virus suspension ex tempore.

In the study of virucidal activity, 3-5 concentrations of DS solution on sterile dechlorinated

tap water at the rate of 1.0 ml of solution for each test.

Staging the experiment.

The contaminated sun is immersed in the solutions under study

tests of 5 pieces per exposure.

The moment of wetting the tests with DS solution is the beginning of the experiment and from this moment the exposure is counted. After 5-15-30-45-60 minutes. Sterile cooled tweezers or loops are extracted for 5 tests, place them in a test tube with beads (glass, plastic) with 5 ml sterile solution of the neutralizer, placed in a joker-apparatus for 10 min.

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After the expiration of each exposure, the wash liquid is contaminated the culture of cells (mice, ducks). Cell culture, washed from growth medium, leave to contact for 30-60 minutes. for the adsorption of the virus. Then cells are washed with Hanks solution and poured with a supporting medium with serum. The cells are incubated in a thermostat at optimal for a particular virus temperature.

The criterion of DS virucidal activity is a decrease the amount of virus not less than 4 $\log_{10}$ with time disinfection time is not more than 60 min.

Control of cell culture.

For this purpose, test tubes with culture are left uninfected cells and observed during the maximum period of the experiment. At work with the cell culture in the control tubes also, as in the experimental, If necessary, the supporting medium is changed depending on the changes in pH.

Control of the virus.

For this purpose, 5 pieces of tests contaminated with the virus, is placed in a test tube with 5 ml of Hanks solution, kept the maximum exposure used in the experiment; transferred to test tubes with 5 ml of neutralizer and beads, shake in a joker-apparatus for 10 minutes. and infect the cell culture (or other sensitive biological object) with the appropriate dose (0.2 ml or less) of the liquid washed from the tests. To find out the quantity a viable virus is titrated.

Nonspecific cytopathic effect (CPE) may be associated with
incomplete neutralization of the disinfectant or cytotoxic effect
mixture "disinfectant + neutralizer". Delete the nonspecific
cytotoxic effect in some cases is possible by

additional washing of the monolayer with Hanks solution after contact
cells with introduced sample with neutralizer for 60 min. for
adsorption of the virus on the cell surface. At the end of this time
add a supporting medium. Avoid incomplete neutralization
you can by carefully working out the conditions of neutralization or search
another neutralizer.

Contamination control of virus tests. To this end, 5 pieces
of the tests contaminated with the virus are placed in a test tube with beads and with
5 ml of physiological solution, washed in a joker-apparatus in
for 10 minutes. and a suitable dose (0.2 ml or less, as in
experience) of the liquid washed from the tests, infect the cell culture (or
another biological object). To determine the degree of contamination
virus tests are performed by titration.

Control of completeness of neutralization of DS.

For this purpose, 5 pieces of tests (without virus) are placed on the
the maximum exposure used in the experiment in 5 ml
solution of the maximum concentration of DS. After this, the tests transfer
for 5 minutes in 5 ml of neutralizer solution, wash with beads in
for 10 minutes and add (0.2 ml or less) to the flushing, from the tests in
cell culture (or other biological object).

Studies of the virucidal activity of a substance are completed
the first stage.

Studies of the virucidal activity of DS intended
for disinfection with especially dangerous viral infections are terminated
the first stage, if the causative agent for resistance to a specific DS is not
exceeds poliovirus and adenovirus.

After receiving data on the presence of DS virucidal

The research activity continues at the second stage - the study of

The effectiveness of disinfection of various objects.

Effective means (substance) providing

inactivation of the virus at exposure time not more than 60 minutes.

1.6.4. Methods of studying and evaluating the virucidal efficacy of DS, intended for disinfection of various objects, contaminated with test viruses

Studies of the effectiveness of DS in the decontamination of various

objects (medical devices, including endoscopes,

dishes, linen, surfaces, excretions, etc.), having

epidemiological significance in the spread of infectious

diseases of viral etiology, are carried out for the purpose of developing

effective disinfection regimes. Received positive

The results in experiments on test viruses are specified (if necessary) by

viruses - causative agents of the infection, in which they will be

are used, given their specificity and mechanism of transmission of infection.

The scope of DS studies depends on their intended use and

can include all or some of the following objects.

1.6.4.1 Determination of the virucidal activity of DS intended for disinfection of medical devices

1.6.4.1.1 Determination of the virucidal activity of DS intended for disinfection of medical devices (except endoscopes) from different materials.

As test products use sterile tools and

other medical products (including single use):

having lock parts (forceps, scissors, corncang), not having,

locking parts (tweezers, spatulas); dental, including

rotating tools (burs, root drills, mirrors, disks

grinding) from various materials (metals, rubbers, glass,

plastics) or simulating their test objects.

Contamination of products with a test virus.

Sterilized or disinfected (by
boiling) test products contaminate the sun with the addition of
40 percent of inactivated (without preservative) SSRCs as
organic load (for example, to 6 ml of a viral suspension
add 4 ml of undiluted serum), and mix. If
means has fixing properties, the serum as
of the protein load is added in an amount of 5%.

The prepared mixture is contaminated with products, completely
immersing in it small, and on large put by a pipette, watching for
uniform wetting of the entire surface. Product channels
filled with a syringe or other device, avoiding
the formation of air bubbles.

The detachable products are immersed in the mixture and, lowering into it, make
several working movements. Tools with lock parts,
immerse open, having previously made them several workers
movements for better penetration of the virus into hard-to-reach
parts of the product in the area of the lock. Excess mixture is removed by any
(blotted with sterile filter paper,
gauze napkins), but do not wipe! products. Test-products
dried at a temperature of 20 ± 2 ° C for 60-90 minutes.

Determination of the active concentration of the agent and time
of a virucidal effect. Contaminated products
immersed in a disinfectant for the duration of disinfection (5, 10,
15, 20, 30, 45, 60, 90, 120 minutes) or, if necessary, more

a long time, for example, 240 minutes. - for single-use products
application before disposal.

At the end of the disinfection time
is extracted from the disinfectant. The channels are washed with a neutralizer,
from surfaces take washings with napkins in the size 5x5 sm, wetted
neutralizer. Napkins, small items are immersed in test tubes with
beads and with a neutralizer (volume - 5 ml), which are placed in

joker-apparatus for 10 minutes.

The resulting flush fluid is introduced into test tubes or wells cell culture tablet or injected into the body of a laboratory animal (or ducks).

The optimal disinfection time is not more than 60 minutes, for single use products - no more than 240 min.

1.6.4.1.2. Determination of the virucidal activity of DS intended for disinfection of endoscopes, including high-level disinfection level of

As the investigated objects use a flexible (gastroscopy), hard (cystoscope) endoscopes or fragments of flexible endoscope (gastroscope) - its channels and the external surface.

Using a pipette, the sun contaminates the surface and channels endoscope (or fragments thereof), then they are immersed in a solution of DS, filling them with product channels. To simulate a minimum organic contamination to the sun before contamination of the test product 5% inactivated serum is added. After 5-60 minutes.
The test article is removed from the solution and a flush is taken from its surface Sterile gauze cloth moistened with a neutralizer. Channel the products are washed with a neutralizer solution and left for 10 minutes.

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Napkins are placed in test tubes with a neutralizer and glass beads, washed in a joker-apparatus for 10 minutes. Washable - from the channels and flushing - from the napkins the liquid is introduced into the cell culture (or infect another biological object), washed from growth medium, leave to contact for 30-60 minutes. for the adsorption of the virus and add a supporting medium. If necessary, for removal cytotoxic effect, DS cells are washed with Hanks solution and fill with a support medium with serum. Cells are incubated in thermostat at the temperature necessary for a particular virus.

The optimal disinfection time is not more than 60 min.

When developing a high-level disinfection regime as a 5% of the inactivated SRRS is used for the organic load. DS

is used in a concentration providing sterilization, and
investigates only the high-level disinfection time.

The optimal time for the TLD is no more than 30 minutes.

1.6.4.1.3 Determination of the virucidal activity of DS intended for disinfection of dental impressions

As a test object, reprints from alginate,

silicone or other materials. For the production of impressions

The impression mass obtained in accordance with the recommendations

are placed in a plastic or metal spoon and

make an impression from plastic dentures with a modeled

gum.

On the impressions, the virus suspension is applied, dried for

2-3 minutes, washed with sterile water and immersed completely in
solution of DS. Control contaminates with a viral suspension

The impressions are immersed in sterile tap water (instead of

solution of DS) for the maximum time taken in the experiment.

After 5-30 minutes. The impressions are extracted from the DS solution (control -

from water) and do washings with a gauze cloth moistened in 5 ml

neutralizer, prepared on a supporting medium.

Napkins are placed in test tubes with a neutralizer and

glass beads, washed in a joker-apparatus for 10 minutes.

Flushing fluid infects the cell culture (or other

biological object). The culture of cells washed off from the growth medium,

leave on contact for 30-60 minutes. for the adsorption of the virus. Then the cells

washed with Hanks solution and poured in a support medium with

serum. Cells are incubated in a thermostat at the optimum for

specific virus temperature.

The optimal disinfection time is no more than 30 minutes.

1.6.4.1.4 Peculiarities of setting up experiments to study virulicide activity of DS intended for disinfection medical products in installations (washing-disinfecting machines, etc.)

One of the most important conditions for setting up experiments on development (for domestic) or expert evaluation (for foreign) disinfection of medical devices in installations, for example, ultrasonic, washing-disinfecting machines, is the creation of experimental conditions, as close as possible to the conditions of practical application (in accordance with the technical passport and operating instructions).

Principal are the following characteristics: speed movement (circulation) of the disinfectant solution; temperature of solution, the quantity (volume) of the solution, the diameter of the channel (s) through which

circulates the solution. The increased temperature of solutions of DS and forced circulation increase their activity, several times reducing the decontamination time of medical devices.

1.6.4.2 Determination of the virucidal activity of DS intended for the disinfection of items for nursing and toys.

Determination of virucidal activity of DS in disinfection items of care for patients.

Subjects of care for patients can be divided into 2 groups in dependence on possible contamination with biosubstrates and the extent contamination by viruses:

1) tips to enemas touch when using them with the mucous membrane of the rectum, so the requirements for their processing and criteria for the effectiveness of DS are the same as for products medical purpose. Organic loading in experiments should be 40% serum, and the method of disinfection - by immersing in the DS solution.

2) underlaid oilcloths can be contaminated by any emissions, so the organic load should be the same, as well as in experiments with medical products - 40%, and the method of decontamination by immersion in solution or single- necessity - two-time wiping.

3) bubbles for ice and warmers touch only the skin, they,
As a rule, they are not contaminated by secretions, however,
are contaminated with viruses. When developing their
disinfection organic load on test objects is not
Required, and the method of treatment - one- or two-time wiping.

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As test objects imitating care items
patients, use a test surface of 10x10 cm,
made of rubber heaters, ice packs, medical
glues, as well as tips for enemas, etc., from different materials.
They are contaminated with a mixture of viral suspension with 40%
inactivated serum. Tips to enemas are contaminated
virus by the same method as medical products,
having channels, then dried until completely dry at
temperature 20 ± 2 ° C.

Prepared test objects are wiped once or
twice with an interval of 15 minutes. a tissue moistened with a solution of DS.
In addition, test objects made from medical adhesive,
immersed in a disinfectant solution.

Small subjects (tips for enemas, etc.) are immersed in
solution DS, filling them with cavities and channels, avoiding formation
air bubbles.

After 30, 60, 90, 120 minutes. washings are taken with gauze napkins
size 5x5 cm, soaked in a neutralizer. Care products,
having channels, are washed with a neutralizer (not more than 5 ml),
which is collected in sterile test tubes and left for 10 minutes. for
neutralization. Gauze napkins are immersed in a wide-necked
tubes with beads (with 5 ml of neutralizer, prepared on
maintenance environment), which are washed in a joker-apparatus in
for 10 minutes. Further - as in 2.1.1.4.

The optimal disinfection time for items of care
patients - not more than 120 min.

Determination of virucidal activity of DS in disinfection
toys.

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When disinfecting toys (small and medium), the definition The virucidal activity of DS is carried out by treatment with methods rubbing, immersion in a solution of DS or irrigation (for large toys).

For this purpose, toys (without holes) are contaminated with a viral suspension at the rate of 0.5 ml per 100 cm^2, but so that all of them the surface was covered with it. Small toys are immersed completely into the viral suspension. Then they are dried at a temperature of 18-20 ° C until completely dry.

The surface of toys is wiped with a cloth moistened with a solution DS (in control - with sterile water), small toys are immersed in solution DS, preventing their ascent; large toys irrigate solution of DS. The rate of consumption of a solution of DS by wiping 100-150 ml / m^2 with a single treatment, irrigation method - 150 ml / m^2 when treated with a Kvazar spray and 300 ml / m^2 - spraying of the "AutoMax" type or a water jet. When need for treatment by wiping or irrigation repeat after 5-15 minutes.

After certain time intervals (from 15 to 120 minutes) using a gauze pad (5x5 cm), moistened with a neutralizer, prepared on a supporting medium or saline solution, from toys take washings. Napkins are immersed in test tubes with glass beads and a neutralizer. Further - as in 2.1.1.4.

The optimal disinfection time is not more than 120 min.

1.6.4.3 Determination of the virucidal activity of DS intended for disinfection of linen, overalls and other articles made of fabrics

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When determining the virucidal activity of DS at

Disinfection of laundry takes into account the ratio of solution of DS and linen:
with extremely dangerous infections, the solution consumption is 5 liters per 1 kg
dry linen, with the other - 4 liters of solution per 1 kg of dry linen;
the temperature of the solution, the degree and nature of the contamination of the laundry.

Determination of the effectiveness of DS in the disinfection of laundry without visible pollution. Studies are carried out using cambric tests. The virus-contaminated tests are dried room temperature, then they are pawned (5 pieces per each) in coarse sterile pouches of 5x8 cm with sewn to the corner each of them a strong thread about 0.5 m long. The bags are closed in the form of an envelope.

Linen (old coiffress gowns, towels, etc.) is immersed in capacity with a solution of DS, consistently soaking one thing over another, making sure that things do not form between things air interlayers that hinder the process of disinfection.
At the same time, the layers of laundry are distributed (top, middle and below) bags with virus-contaminated tests.

After certain time intervals (15-30-60 and more minutes)
The bags with the tests are extracted simultaneously from three layers.

Tests are removed from the bag with sterile tweezers, immersed in wide test tubes with a neutralizer and beads, then - as in p. 1.6.4.1.4.

Determination of virucidal activity of DS in disinfection linen, contaminated with blood, feces. To study effectiveness of the means for decontaminating laundry, contaminated blood (imitation in the form of 40% serum), medical waste from fabrics, gauze, cotton wool (disposable surgical underwear and obstetric

kits, dressings, gauze napkins, cotton wool tampons, etc.) to 6 ml of a virus-containing liquid are added 4 ml inactivated by the SRRS, mix and fill in the tests, dry they are used in the experiment (Section 1.7.4.1.3.).

The optimal time for disinfection of the laundry is not more than 120 minutes. medical waste - not more than 240 min.

To simulate the contamination of laundry and medical waste from tissues and other feces in experiments with the test virus are used fecal emulsion. Fecal emulsion is prepared as follows way: to 6 ml of 10% BC add 4 ml of 40% emulsion of feces. For this 8 g sterilized feces (1.5 atm for 30 min.) grinded in a mortar with 20 ml of sterile water. As an organic 80% of the inactivated SRNC from Calculation: 8 ml of serum and 2 ml of viral suspension, carefully mix and fill this mixture with tests. Excess fluid through 15 minutes. remove by pipette, the tests are dried room temperature. The experiment is then carried out according to the scheme for uncontaminated laundry (clause 1.7.4.1.3.).

Optimal time for disinfection of clothes and medical waste contaminated with feces - no more than 240 min.

1.6.4.4 Determination of the virucidal activity of DS intended for disinfection of dishes, including laboratory

The technique of disinfecting dishes without food debris, and also without other visible contaminants.

When disinfecting dishes without food, laboratory dishes with no visible impurities as test objects use plates, glasses, enamel mugs; cutlery - knives,

forks, spoons; laboratory glassware - pipettes, incl. micropipettes, tips to them, slides, test tubes.

Clean dishes are contaminated with a virus suspension, which are pipetted at a rate of 0.5 ml per 100 cm^2 and uniformly distributed Apply a sterile glass spatula over the surface. Forks, spoons and the knives are immersed in a viral suspension for 10-15 minutes. (behind excluding handles), the pipetal channels are contaminated with the virus.

Crockery and cutlery are dried at room temperature temperature. After complete drying, immerse in a solution of DS, completely covering them (the consumption of the solution is approximately 2 liters per set of dishes: cup, saucer, 2 plates, spoon, fork, knife)

solution.

At certain time intervals (15, 30, 60, 90, 120 minutes) the dishes are removed from the DS solution. To assess the effectiveness disinfection with sterile gauze napkins measuring 5x5 cm (initially moistened with a neutralizer, then dry) carefully wipe the virus-contaminated parts of each object. Napkins are placed in a sterile, wide tube with beads and with 5 ml sterile neutralizer (1.6.4.1.4.).

The optimal disinfection time is not more than 60 min.

The control serves in the same way as the contaminated virus, utensils immersed in the maximum exposure in sterile or boiled tap water.

The method of disinfecting dishes with food remains, as well as contaminated laboratory dishes. To study the effectiveness of DS when disinfecting dishes with food residues polluted laboratory reusable and single-use of the application (before disposal) to the viral suspension, 80%

inactivated SRRS at the rate of: 20% of the viral suspension and 80% Serum, the mixture is applied evenly on the dishes, dried. Further - as in Section 2.4.1.

The optimal disinfection time is not more than 120 min. for reusable utensils and no more than 240 min. - for dishes single use (before disposal).

1.6.4.5 Determination of the virucidal activity of DS intended for disinfection of surfaces

Determination of virucidal activity of DS in disinfection Test surfaces are studied in two ways: by rubbing (one- or two-fold) or irrigation method.

In the experiments, a test surface of size 10x10 cm, smooth, rough, absorbent and non-absorbent DS from various materials: wooden, plastered, painted oil or glue, etc. paints, pasted wallpaper, as well as from linoleum, plastic, glass, tile, metlakh tile, faience,

artificial or natural leather, etc. A set of test surfaces
(hereinafter - "surface") is determined by the purpose of the facility.

Before the experiment, the surfaces are subjected to a mechanical
clean - wash with soap and water, except for
The surfaces pasted with wallpaper and painted with glue paint.
The latter are wiped several times with a sterile napkin,
moistened with sterile tap water. After drying
surfaces are placed horizontally and pipetted with a viral
suspension at a rate of 0.5 ml with the addition of 5% of the activated SNRS
on an area of 100 cm^2, evenly distributed over the surface
glass spatula. Contaminated virus surfaces

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dried until completely dry at a temperature of 20 ± 2 ° C and
relative air humidity of 50-60%, then treated
solution of DS.

When disinfection of contaminated surfaces
some types - plastered, painted glue, etc.
paints, pasted with wallpaper, made of glass, faience, wood, painted
oil paint, from tile, have a vertical
treatment in this position (irrigation method). Rest
The surfaces are treated as in a horizontal (single-shot
or double wiping), and in vertical positions.
The DS solution is applied to the surface by spraying from an atomizer,
precisely following the amount of liquid consumed. Norm
flow rate - from 80 to 500 ml of solution per 1 m^2 of treated area.

To simulate organic pollution, 40%
inactivated serum in the development of disinfection regimes
shells, baths or in the development of disinfection of toilet bowls -
80%. It is also possible to use a virus-containing fecal
emulsion, which is applied on the surface of tiles or faience in
experiments with viruses that are excreted from the body with feces.

The efficiency of decontamination is controlled through
15-30-60 min. Samples are selected by thorough wiping
Irrigated with a solution of DS surfaces slightly moistened

neutralizer in physiological saline or Hanks solution with antibiotics with a sterile gauze cloth (5x5 cm), and then dry. Napkins are placed in wide-necked tubes with beads with 5 ml neutralizer (see 1.6.4.1.4.).

Optimum disinfection time for surfaces - no more than 60 min.

In the control of the virus, contaminated sun surfaces wipe or irrigate sterile or boiled tap water at the same rate of water discharge as the DS in experience. Sampling and processing are carried out similarly to the experimental ones. For To determine the contamination density, titration of washings with surfaces.

When working with the hepatitis A virus, experience is simulated with less the amount of laundry, DS and viral suspension.

1.6.4.6 Determination of the virucidal activity of DS intended for decontamination of excreta (urine, feces, sputum) and blood

When determining the virucidal activity of DS for Disinfection of urine is selected concentration of DS and time processing. DS solutions are added to the disinfected process boiling urine in equal or double volume. After 15, 30, 60 minutes. this mixture in an amount of 1 ml is transferred into tubes with 5 ml neutralizer, stirred, left for 10 minutes. for neutralization, then infect the cell culture (or other biological object). The cell culture, washed from the growth medium, leave on contact for 30-60 minutes. for the adsorption of the virus. Then the cells washed with Hanks solution and poured in a support medium with serum. Cells are incubated in a thermostat at the optimum for specific virus temperature.

In the control to urine add not a disinfectant solution, but sterile water. The results of the experiments are taken into account in comparison with control. The amount of the virus in the urine in the control is determined titration.

The optimal disinfection time is not more than 120 min.

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In the development of disinfection regimes, feces are taken into account ratio of DS and decontaminated mass of feces, treatment time, the consistency of disinfected emissions, the degree of homogenization in process of disinfection.

To this end, 20 g of sterilized faeces are ground in mortar with the addition of 80 ml of water until a homogeneous emulsion is obtained. The emulsion is poured into 9 ml into tubes and 1 ml virus-containing liquid.

The prepared emulsion of feces is filled with equal or with a double amount of DS solution, and subsequently take samples in the same way, as in the case of disinfection of urine. Taken samples are centrifuged at 2500-3000 rpm. for 20 minutes, after which the supernatant the cell culture by injecting it into test tubes / wells with cells, or in the body of a sensitive animal, ducks.

In the control, instead of the DS solution, sterile water is used. The results are taken into account in comparison with the control.

The optimal disinfection time is not more than 240 min.

When developing regimes of blood disinfection (without clots) use citrate (or defibrinated) blood, contaminated with the test virus (blood volume and virus suspension 1: 1). You can also use erythrocyte mass (lamb erythrocytes), which is prepared by adding to 3 ml washed erythrocyte mass 97 ml 3.0% albumin solution on phosphate buffer. Aliquots of this mixture contaminate test-virus. The optimal ratio of DS and disinfected blood, concentration of DS, treatment time, neutralized (1: 1, i.e., 1 volume of a mixture of blood and DS and 1 volume of neutralizer) withstand 5-10 minutes. (stirring or shaking) and used to determine

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infectivity of the test virus. For disinfection of blood with clots
Only the thermal treatment method is effective (in the steam
sterilizer).

The optimal disinfection time is not more than 240 min.

When developing sputum disinfection regimes,
sputum of volunteers, select the optimal ratio of DS and
disinfected sputum, time of treatment, concentration of DS,
neutralizer.

The optimal disinfection time is not more than 240 min.

1.6.4.7. Determination of the virucidal activity of DS aerosols, intended for air disinfection in premises

To determine the virucidal activity of aerosols DS at
disinfection of air in the premises (infectious foci,
preventive institutions, etc.) use the aspiration method.

The test viruses are poliovirus and / or
adenovirus. The air is sprayed in the chamber in an amount sufficient for
obtaining in the air of the chamber the concentration of the virus $1x10^5$ $TCDD_{50}$ / m^3 .
Aerosol is created using spray equipment, which
ensures the formation in the air of not less than 80% of the particles with
dispersibility of 20 + 5 μm, then include a fan for
prevention of DS aerosol settling.

In Drexel flasks instead of 50 ml of sterile tap water
pour 5 ml of Hanks solution or maintenance nutrient
environment with a neutralizer and antibiotics.

To control the initial air contamination of the virus before
the beginning of the experiment through Drexel's flasks, connected
one after the other, and also during the experiment

pass 50 liters of air (sample volume for the study). After
sampling every 5, 10 or 15 minutes, depending on the
estimated efficacy of DS and taking into account the sensitivity of the virus
to DV, a liquid from two Drexel flasks is connected, mixed and
2 ml are added to test tubes / wells with a culture of cells (or into the body

laboratory animal, ducks). The cells are left for 1 hour for contact, then poured. After this, all test tubes are introduced maintaining medium in an amount of 2 ml and placed in a thermostat.

When determining the effectiveness of aerosol DS of the study are carried out at three parameters of relative humidity: 20-25%, 50-55% and 80-85% and an air temperature of 20 ± 2 ° C. During experiment in the chamber, the fan must be constantly operated mixing of the components of the investigated system - virus, air, aerosol DS.

On the inactivation of the virus is judged by the loss of infectious activity.

The virucidal activity of DS in the form of an aerosol depends on concentration of virus in air, consumption of aerosol mixture per unit volume, concentration of DS solution in the aerosol mixture, exposure, relative air humidity and temperature in the chamber.

In the control, aerosol mixtures containing solution DS sterile water, which are sprayed in the chamber in the same quantities, while the size of the aerosol particles must be the same as the particle size of the aerosol of the agent.

1.6.4.8. Determination of the virucidal activity of DS intended for disinfection of medical waste

Methods for determining the virucidal activity of DS at disinfection of medical wastes of different origin are given in the relevant sections - medical devices appointment of a single application (Section 1.7.4.1.1.); waste from tissues (medical protective clothing and linen, dressings, gauze napkins, cotton swabs, etc.) (clause 1.7.4.3.); dishes, including number of laboratory single use (1.7.4.4.); blood and (Section 1.7.4.6.).

1.6.5. Determination of the virucidal activity of antimicrobial tissues, paint and varnish materials

In determining the virucidal activity of antimicrobial
the choice of a test-virus and a test object depends on the purpose and scope
use, for example, gauze bandages to protect the upper
respiratory tract of a professional contingent (medical
workers, sellers in supermarkets, etc.) or manufacturing
overalls, etc.

To this end, for test objects (two for each dilution) from
of the test tissue 2 × 2 cm in size, drop by drop, 0.05 ml
viral suspension. After 30 seconds, 1, 3, 5, 10, 15, 30, 60 minutes. test-
objects are transferred to broad-necked test tubes with beads and with 5 ml
neutralizer, washed in a joker-apparatus for 10 minutes.
Flushing fluid infects the cell culture (or other
biological object). The cell culture, washed from the growth medium,
leave on contact for 30-60 minutes. for the adsorption of the virus. Then the cells
washed with Hanks solution and poured in a support medium with
serum. Cells are incubated in a thermostat at the optimum for

specific virus temperature. In the control, test objects are used
from the tissue of the same article, but not containing antiviral
substances.

Criteria for virucidal activity - a decrease in the virus titer is not
less than 4 $\log_{10}$.

Definition virucidal activity paint and varnish
materials. When determining the virucidal activity of paint and varnish
materials (antimicrobial varnishes, paints) a viral suspension in a dose
10^5 $TCDD_{50}$ / ml is applied in an amount of 0.5 ml to the test-
surface (size 10x10 cm). After the necessary exposure
(from 30 minutes to 24 hours) from the surface take a wash with a sterile gauze
a tissue moistened with a neutralizer. Wipes are immersed in
wide-necked test tubes with 5 ml of neutralizer and beads, washed in
for 10 minutes. in the joker-apparatus (Section 1.7.4.1.4.).

As a control, test surfaces, painted
paint or lacquered, do not contain virucidal substances.

Determination of prolonged virucidal action

paint and varnish materials are carried out on test surfaces without

additional and with additional artificial contamination

tested test virus.

Criteria for the virucidal activity of the paint coating -

decrease in the amount of the virus by at least 4 log $_{10}$ within 24 hours after

applying it to the surface; observation can continue in

for 6 months or more.

1.6.6 Appendices

Annex 1. (recommended)

1. Test viruses and methods for their cultivation

1.1. Test viruses

Test viruses are obtained from national or international

virus collections:

1.1.1. A type 1 poliomyelitis virus (the Sabin vaccine strain

(LSc-2ab), an RNA virus containing no envelope from the family

picornaviruses;

1.1.2. Adenovirus, type 5, strain Adenoid 75 (ATCC VR-5),

A DNA virus containing no envelope from the family

adenoviruses;

1.1.3. Bovine parvovirus, strain Haden (ATCC VR-767);

1.1.4 Hepatitis A virus, strain HAS-15, RNA-containing virus,

not having a membrane, from the family of picornaviruses;

1.1.5 Hepatitis B virus in ducks (HBVU), strain "UFA-04",

DNA-containing, from the family of hepadnaviruses;

1.1.6. Human hepatitis C virus, strain D1;

1.1.7 Bovine diarrhea virus (VD-BS), an RNA-containing virus

Diarrhea - diseases of mucous membranes of cattle, from

family of pestiviruses, strain VK-1B1-No. 28.

1.2. Cultivation of viruses

1.2.1. "Source Virus" is a virus derived from a reference centers (from national or international collections of viruses), multiplied in volumes sufficient for long-term work with given passage, with minimal amounts of passages. Stored

in small volumes at -70 ° C or in liquid nitrogen (Guideline on virological studies of poliomyelitis, WHO, Geneva, M., 1998).

1.2.2. "Test viral suspension" is a viral suspension, The virus obtained from the original virus and used to determine viricide properties of the disinfectant.

1.2.3. The original virus is propagated in sensitive cells (laboratory animals, ducks, etc.) that produce the virus in high titles. Cell detritus is removed by centrifugation at low revs (1000 rev / 5 min.). This material is called "test-virus suspension. " It is used not divorced.

It is assumed that the minimal titer of the viral suspension according to at least 106.5 TITD $_5$ 0 / ml. In any case, it should be high enough so that you can get a drop in the 4.0 log $_{10}$.

Poliovirus is propagated in a transplantable cell culture of RD and HEp-2 or in other sensitive cell cultures (4647, Vero and etc.). To obtain BC, a cell culture infected with a virus poliomyelitis at the stage of 100% damage to the monolayer caused by cytopathic action of the virus, triply frozen and thaw. After removal of the destroyed cells by centrifugation, the resulting suspension is used in experiments.

Adenovirus is propagated in a transplanted cell culture HEp-2, 4647 and in other sensitive cell cultures. Methodology obtaining the aircraft is similar to the above, except that before the procedure of cell destruction by the freezing-

thawing, the culture medium is replaced by a non-containing serum and add it in a smaller volume.

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Hepatitis A virus is propagated in a transplantable cell culture 4647. To obtain a viral suspension, the vials are incubated at 37 ° C for 21-28 days with a weekly change of environment. After the incubation is over, the maintenance medium is removed from the vials, the monolayer was washed with Hanks solution, warmed up to 37 ° C Vershen's solution, the monolayer was washed with a dispersant, a solution Versene is drained and 0.1M phosphate-buffered saline is added, pH 7.6.

The infected cells are resuspended in phosphate buffered saline, the cell suspension is frozen fivefold (-70.degree. C.) and thawed under room temperature. The resulting cell lysate containing 6.5-7.0 $\log_{10}$ the hepatitis A virus is homogenized and clarified centrifugation (3000 rpm), then used as a test virus.

The supernatant is evaluated for antigen content of viral hepatitis A by the method of enzyme immunoassay. Optical The density (ОП450) of the test samples treated with DS should correspond to OP450 control samples that do not contain a virus hepatitis A.

The technique for determining infectious titers is based on detection of the hepatitis A virus antigen by titration of the final dilution. Prepare 10-fold dilutions of the analyzed material on medium Eagle MEM x 2. By dilution of the virus, beginning 10^3 infect no less than 4 identical containers with dense monolayer of cell culture 4647. After incubation for 21-28 days. The supernatant is evaluated for the content of the viral antigen method of enzyme immunoassay. Infectious titres are calculated by the method of Reed and Mench or by the Sperman-Kerber method (Annex 3).

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Hepatitis B virus in ducks The method of modeling HBGV infection in vivo.

The source of the virus in modeling the infection is blood serum of a domestic duck infected with HBGV in vivo when Experimental infection with a virus with known infectious properties. For the HBGV test virus used in each of the evaluation of DS, an infectious titer should be determined.

An important point in the preparation of experience in testing the DS with using the modeling of HLV-infection in domestic ducks is determination of the number of experimental groups. For reason widespread prevalence of natural HBV-infection and There is no screening for HBGV in embryo supply farms and ducklings in the territory of the Russian Federation before laying down the experience It is necessary to pre-test for HBHV DNA for participation in the experience of ducks with a natural infection. In view of the maximum susceptibility to infection with HBHV ducklings aged 1-3 days It is advisable to work with ducklings of this age, however preliminary screening may lead to a delay in the experiment, and, as a consequence, to a decrease in the number of susceptible to infection individuals. Therefore, it is recommended to increase the experimental groups up to 10-15 goals each, taking into account the potential number of ducklings with natural HBV-infection, taking blood from all ducklings immediately before the introduction of the experimental material, the definition of DNA of HBGV for a short time and the elimination of ducklings from revealed by natural HBV-infection.

After definitions number of experienced groups conduct directly processing the DS virus. DS processing should be carried out immediately prior to the introduction of material to ducks,

the need for DS treated viral preparation can be stored not more than 24 hours at +4 ° C without reducing potential infectious properties.

Treatment of the DS virus is carried out according to the standard procedure, with

using a suspension method or the method of bathist test-objects.

Then, neutralization of the DS is carried out according to a standard procedure. Three-day ducklings are placed on 10 heads in isolated cages on number of groups of the experiment. Then all ducklings take blood, and use for subsequent PCR analysis. After taking blood ducks intraperitoneally injected material obtained as a result of processing VGVU DS. It is shown that intraperitoneal infection of HBGV as effectively as intravenous, but it is less traumatic for animals. Each duck is injected with 200 μl of material, in each pilot group use the appropriate material treated selected concentration of DS. In the positive and negative groups the corresponding control material is administered in the same way.

After receiving data on the detection of natural HBGV-infection among the ducks involved in the experiment, positive for The HBVU of the individuals is removed. All the ducks participating in the experiment take a blood sample 3 weeks after infection. Take blood and the separation of blood serum are carried out in the same way as when pre-tested for natural HBV-infection.

In the selected samples, the determination of HBV-infection in PCR is carried out. The evaluation of the experimental results is carried out using the "yes / no" system, then there is the appearance of even one case of HBV-infection in the experimental group is regarded as the infection of HLRN, and, as a result, indicates

on the insufficient virucidal properties of the analyzed concentration DS.

Diarrhea virus - diseases of mucous membranes of large horned Cattle are multiplied in the cells of the KST-transplantable cell culture coronary vessels of the fetal heart of the cow. There is a virus in the museum «All-Russian research institute experimental veterinary science named after Ya.R. Kovalenko ».

The human hepatitis C virus is multiplied in a cell culture by method developed in the GU Research Institute of Virology. DI. Ivanovsky RAMS.

Annex 2. (informative)

Statistical processing of results

A. Determination of a 50% dose by the method of Reed and Mench and by the method Sperman-Kerbera

Titration of the virus in test tubes and in animals provides for the determination of the dose at which the action of the virus (cytopathogenic effect or death of animals) is manifested in 50% test objects (vitro cultures or animals), the so-called TCDD $_{50}$ or LD50. Since in most cases, according to titration at once it is not possible to determine the 50% dose, The need for statistical processing of results. results can be considered statistically reliable if observed following conditions:

the number of test objects infected with one dilution of the virus, must be at least 4;

titration should include two dilutions of the virus below 50% of the dose and two dilutions of the virus above this dose.

Among the different methods of counting TCD50 or LD50 The method of complete cumulative Reed and Mench and the method of determining the "central magnitude" of Kerber.

1. The Reed LJ method, Muench HA A simple method of estimating 50% endpoints. Am. J. Hyd., 1938, 27, 493-497. Simplified variant according to the book by Voroshilova MK, Zhevanderova VI, Balayan M.S. Methods of laboratory diagnosis of enterovirus infections. M., Medicine, 1964. - p. 127-129. Statistical processing of results).

The method is based on the logical premise that the tissue culture or an animal that died while infecting them with some kind of breeding virus, will die and when infected by any lower dilution.

An example of counting is shown in Table. 1.

Table 1

50% dose calculation (TCDD $_{50}$) according to the method of Reed and Mench

Intelligence The	16 officer honor	Initial perish data	survived	died	Cumulative data doom

virus	test-an object	about	survived		Percent
10 -5	4 - 4	- 0	7th	0	100
10 -6	4 - 2	- 2	3	2	60
10 -7	4 - 1	- 3	1	5	17th
10 -8	4 - 0	- 4	0	9	0

From Table 1 it can be seen that a 50% dose is between the dilutions virus 10 -6 and 10 -7 .

Then calculate the value of X, which must be added to Dilution directly below 50% of the dose (in log 10) is performed by the following formula:

$$X = \frac{A - 50}{BA}, \qquad (1)$$

Where:

A - percentage of death at breeding, directly below the desired 50% dose (in this case 60%);

B - percentage of death at breeding is immediately higher the desired 50% dose (in this case, 17%).

Substituting these values into formula 1, we find:

$$X = \frac{60 - 50}{60 - 17th} = , 23.0$$

from where the virus titer (in inverse log 10) is 6 + 0.23 = 6.23; other in words, one TCDD 50 or LD 50 corresponds to the dilution of the virus 10-6.23.

If the titration was taken dilutions of the virus at an interval 0.5 log 10 , then the value of X in formula 1 should be multiplied by 0.5.

Since titration of the virus in the vitro cultures usually produce clear results and cultures with 100% degeneration are separated from cultures with complete absence of degeneration only one dilution of the virus, it is convenient to use in the counting titles by Reed and Menchu by a simplified scheme:

amount - Amount Percent Titer

test tubes cultures, infected by dilution of 10- n	cultures with CPE	cultures with CPE	virus in log 10
4	1	25	(n-1), 66
4	2	50	n, 0
4	3	75	n, 33
4	4	100	n, 50

for the calculation of TCDD 50 (the amount of cytopathogenic doses) of the virus in 1 ml of the test fluid to the value of the virus titer in log 10 is added depending on the amount of virus-containing

material taken for the infection of one culture, the relevant correction values:

Volume of material in milliliters taken to infect one culture	Amount of correction in log-rhyme
0.2	0.7
0.25	0.6
0.3	0.52
0.4	0.4
0.5	0.3
0.6	0.22
0.7	0.16
0.8	0.1

For example, if in all test cultures contaminated with 0.2 ml of the material in a dilution of 10 -4 , a cytopathogenic effect, and in cultures inoculated with 10 -6 dilution , cytopathogenic effect is not observed (with inoculation 10 -5 cytopathogenic effect was noted in one of four vials), then the virus titer in log 10 will be 104.66. The content of his in 1 ml will be equal to 105.36 TCDD 50

2. Determination of TIDC 50 of the virus using the Sperman-Kerber method

TTSD 50 is a negative decimal logarithm

The highest used concentration of the virus, multiplied by logarithm of breeding, that is (the sum of the% of affected cells in each dilution (100 - 0.5) x log10 dilution.

table 2

Example of a titration result

Dilutions, -log 10	Results from the CPD *	Percentage (%) of crops with the presence of a CPD

- 4	4, 4, 4, 4, 4, 4	100
- 5	4, 4, 3, 4, 4, 4	100
- 6	4, 4, 3, 3, 0, 0	66.7
- 7	4, 2, 0, 0, 0, 0	33.3
- 8	0, 0, 0, 0, 0, 0	0
	Amount% with JVC	300

Note: *) - from 1 to 4 - plus system of the% -th expression cell death (1 + = 25%, 2+ = 50%, 3+ = 75%, and 4+ = 100%), 0 = no CPR.

The formula for calculating $TCDD_{50}$ from these data:

-4 - [{(100 + 100 + 66.7 + 33.3 + 0) / 100} - 0.5] x 1 = -4 - [300 / 100-0.5] x 1 = -4 - 2.5 = 6.5.

Thus, the $TCDD_{50}$ will be $10^{-6.5}$ or 6.5 $\log_{10} TCDD_{50}$

3. Method of plaques

To calculate the average number of plaques used the following formula:

$$PFU / t = \frac{\Sigma c_1 + c_2 \ldots . c_n}{n_1 + n_2 x v_2 + \ldots n_n x v_n) x d}$$

Where:

t is the volume added to the well at each dilution step;

c_1 - number of PFE in all wells of the smallest dilution, with which can be accounted for (no longer plum plaques);

c_2 - number of PFE in all wells of the next dilution;

c_n is the number of PFE in all the wells of the last dilution;

n_1 - the number of all wells of the smallest dilution with draining plaques (corresponds to C_1);

n_2 - number of all wells of the second dilution with non-draining plaques (C_2);

v_2 is the dilution factor between n1 / n2 (for example, $n_1 = 10^{-3}$ and $n_2 = 10^{-4}$, then $v_2 = 0.1$);

n_n is the number of all the last dilution wells at which there are plaques (c_n);

v_n is the dilution factor between n_1 and n_n (for example, $n_1 = 10^{-3}$ and $n_n = 10^{-6}$, then $v_n = 0.0001$);

d is the dilution step from 1.

Calculations are performed with integers if the last number less than 5, then it remains unchanged; if it is greater than 5, then rounded to the next whole number.

Example.

$$PFU / t \quad \frac{(52 + 48 + 49) + (25 + 27 + 31) + 5 + 6 + 7)}{[3+ (3x\ 0.1) + (3\ x\ 0.01)]\ x\ 10^{-3}} = \frac{250}{3.33x\ 10^{-3}} = 75075{,}075 = \lg 4.875 = \lg 4.9$$

If the volume added at the dilution is 0.2 ml, then BOE \ ml = $\log_{10}$ 5, 6.

1.7 Methods for studying and evaluating sporicidal activity disinfecting and sterilizing agents

1.7.1 General

Studies of sporicidal activity of substances, DS,
sterilizing agents (hereinafter - SS) and the effectiveness of their regimes

Applications include:

selection and preparation of test microorganisms in a spore form for

study of sporicidal activity of DS, CC and their substances;

ensuring the standard of research conditions

sporicidal activity of DS, CC and their substances;

methods of research and evaluation of the results of sporicidal

activity of DS, CC and their substances in vitro (suspension method,

method of bathist test objects) and the spectrum of sporicidal activity;

methods of research and evaluation of sporicidal efficacy of DS

when developing regimes for disinfection of environmental objects,

contaminated test microorganisms in a spore form;

methods of studying the sporicidal efficacy of CC at

the development of sterilization regimens for medical devices, including endoscopes;

methods research sporicidal activity MOP,

intended for TLD endoscopes.

1.7.1.1 Test microorganisms for the study and evaluation of sporicidal activity of DS, SS and their substances.
Requirements for test microorganisms.

When studying the sporicidal activity of DS and their substances in

quality of test microorganisms use:

Bacillus cereus, strain 96;

Bacillus subtilis, strain 7;

anthrax living dry STI-1 vaccine for humans;

Bacillus anthracis, strain 81/1 (pX01 +, pX02 +), or strain 27

(pX01 +, pX02 +).

When studying the sporicidal activity of CC and their substances in

quality of test microorganisms use:

Bacillus cereus, strain 96;

Bacillus subtilis, strain 7;

Bacillus licheniformis, strain G BKM B-1711D;

Geobacillis stearothermophilius, strain VKM B-718.

Test microorganisms are selected depending on the active substance and from the appointment of a sporicidal DS, CC and its substance.

Requirements for test microorganisms.

Test microorganisms should have typical morphological, cultural, biochemical properties inherent in this species (see Appendix 1), and have standard resistance to reference DS and CC: chloramine 10%, hydrogen peroxide 6%, glutaraldehyde 2.5% (pH 7.2), dry hot air at 160 ± 3 ° C, water flowing steam at 100 ° C, water saturated steam under excess pressure at 121 ± 1 ° C.

Indicators sustainability test microorganisms to The above listed facilities are listed in Table 5.8.

Museum strains of test microorganisms are stored in the refrigerator at a temperature of 6 ± 2 ° C in the form of dry culture in ampoules (after lyophilic drying) no more than 2 years or at a temperature of minus 70 ° C.

Working cultures are stored on sloping wheat agar in refrigerator at a temperature of 6 ± 2 ° C.

Test microorganisms that do not possess this resistance, are subject to replacement.

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The sporicidal activity of DS, CC and their substances determines, using test microorganisms in a spore form.

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T

Stability of spores of test microorganisms to standard disinfectant and sterilizing agents

Test culture		Time of death of test microorganisms, min (min), under action					
Name and strains	to the dispute in test object	chloramine 10%	peroxides hydrogen 6%	glutaric aldehyde 2.5%	dry hot air (160 ± 3) ∘ C)	water flowing couple (100 ∘ C)	water rich couple redundan pressur (121 ± 1
Test microorganisms for the study and evaluation of disinfectants and their substances							
Bacillus cereus, strain. 96	(1-5) · 10 6	360	60	60	-	6-7	-
Bacillus subtilis, strain. 7th	(1-5) · 10 6	360	60	180		6-7	-
Sibiriazvennaya live dry vaccine STI-1 for people	(1-5) · 10 6	360	60	60	-	6-7	-
Bacillus anthracis, strain. 81/1 or 27	(1-5) · 10 6	360	60	60	-	6-7	-
Test microorganisms for the study and evaluation of sterilizing agents and their substances							
Bacillus cereus, strain. 96	(1-5) · 10 6	360	60	60	-	6-7	-
Bacillus subtilis, strain. 7th	(1-5) · 10 6	360	60	180	-	6-7	-
Bacillus licheniformis G BKM B-1711D	(1-5) · 10 6	-	-	-	thirty	-	-
Geobacillis stearothermophilius, strain. VKM B-718	(1-5) · 10 6	-	-	-	-	-	1

The use of *B. cereus* and *B. subtilis* as test microorganisms in the study of sporicidal activity of disinfectant and sterilizing means harmonized with the European standard. Based on the data in Table 5.8. further studies are carried out using the most stable test-microorganism to the test medium.

1.7.1.2. Method for preparation of spore suspension of test microorganisms

To obtain test microorganisms in the spore form, they are
are grown on the nutrient media listed in Table 5.9.

Table 5.9.

Nutrient media for growing test microorganisms in a spore form

№ p / p	Test culture	Nutrient media
1.	Bacillus cereus, strain. 96	Wheat agar
2.	Bacillus subtilis, strain. 7th	
3.	Bacillus anthracis, strain. 81/1, 27	Wheat agar or Hottinger agar with Amine nitrogen 120 mg%
4.	Bacillus licheniformis, G VKM B-1711D	Wheat agar or potato-peptone agar
5.	Geobacillis stearothermophilius, strain. VKM B-718	

List of ingredients and method of preparation of nutritious
media for the cultivation of test microorganisms for
study and evaluation of sporicidal activity of DS, CC and their substances,
are given in Appendix 2.

The process of preparing a test-microbe spore suspension,
used in the study and evaluation of sporicidal activity of DS,
MOP and their substances, includes three consecutive stages:

1) obtaining broth culture of museum
lyophilized or agar culture of the test microorganism;

2) obtaining a spore agar culture of the test microorganism;

3) preparation of a test-microbe spore suspension and evaluation
compliance with its requirements.

When using a lyophilized spore culture
B. cereus and B. subtilis ampoules with these test microorganisms
are opened under aseptic conditions as follows: a tampon

wool, moistened with ethyl alcohol, treat the surface
ampoules, then heat its soldered end over the flame.

To the heated end of the ampoule, put a cotton plug,
moistened with sterile water to create a crack on the ampoule.
A metal tool (a scalpel, tweezers) is chopped off
crack the end of the ampoule. After this, a sterile Pasteur pipette
in the ampoule pour 0.2 ml of sterile drinking water, cover
sterile gauze and leave for 30 minutes. at room temperature
temperature. To obtain a spore suspension, the contents of the ampoule
are mixed with a sterile bacteriological loop.

The thus obtained suspension of spore test-
microorganism is aspirated with a sterile Pasteur pipette and
Transfer 1-2 drops in two tubes with 5 ml of nutrient broth
(Hottinger broth, dry nutrient broth - SPB, meat-peptone
broth MBP) containing 0.5% glucose.

When working with an agent of anthrax for cooking
Spore suspensions of the ampoule with dried cultures are opened in
the museum (collection) of living cultures. Manipulations are carried out
in the biosafety box. At the same time, the drawn end
Ampules are heated above the flame of the gas burner; then wet
end of the sterile cotton swab touch the heated part, in
resulting in cracks. The end of the ampoule is covered
a three-layer gauze cloth moistened with disinfectant
solution and well wrung out, and break off with tweezers.

After opening, the ampoule remains covered with the same tissue in
during one or two minutes. Then the napkin is carefully removed and together
with the remains of the glass immersed in a disinfectant solution. Opened
The ampoule is covered with a sterile gauze pad for 1-2 minutes. Then in

Ampoule is introduced 0.2 ml of sterile drinking water for cooking
spore suspensions, which are further sown in liquid nutrient media,
as mentioned above.

G. stearothermophilius cultures are incubated at a temperature of
55 ± 1 ° C, B. cereus, B. subtilis, B. anthracis and B. licheniformis - when
temperature 37 ± 1 ° C for 24-48 hours.

The broth cultures of test microorganisms of bacteriological

loop or a Pasteur pipette (1-2 drops each) is
test tubes on a sloping nutrient medium (dry nutrient agar -
SPA, meat-peptone agar - MPA). The crops are incubated for
24-48 hours, as indicated above.

To obtain spores of B. licheniformis cultures, G. stearothermophilius
in test tubes with sowing the test-
5 ml of sterile distilled water are added to the microorganism and
wash away the culture that has grown on a solid nutrient medium.
The resulting suspension is transferred to vials or containers up to 250/500 ml,
containing respectively 100/200 ml of the corresponding
test microorganism of a slanted solid (agar) culture medium
(see table 5.9.).

On the surface of the culture medium in each vial (mattress), in
depending on their capacity (250/500 ml), a suspension is introduced,
washed with 1-2 test tubes with crops. Weigh the wiggle of the vial
(mattress) is evenly distributed over the surface of the medium, closed
cotton-gauze stoppers and paper caps and incubated
at a temperature of 55 ± 1 ° C (G. stearothermophilus) or 37 ± 1 ° C
(B. licheniformis) for 10-12 days in an inclined position (under
angle 45 °) agar up. To create sufficient humidity in

Thermostat, operating at a temperature of 55 ± 1 ° C, is placed open
containers with water (up to 2 liters per thermostat with a capacity of 80 liters).

Test-microorganisms of B. cereus, B. subtilis and B. anthracis for
obtaining a spore form is inoculated on sloping wheat agar
or Hottinger agar with amine nitrogen 120 mg% and grown at
temperature 37 ± 1 ° C two days in a thermostat, and then another 7-12 days at
temperature 20 ± 1 ° C in a dark place. On days 7 and 9 of the culture
check for the intensity of sporulation. For this purpose,
2-3 bottles (mattresses) culture is taken from the upper, medium
and the lower parts of the agar; all three samples are triturated together on one
glass, spreading a thin layer. A smear is fixed over
flame burners, painted on Gram or gentsianvioletom (on
Sinev). Painted preparations are washed with drinking water,

dried and microscopized with an immersion system - spores
have the appearance of unpainted voids within the cells.

When working with B. anthracis for fixing smears, 90%
Ethyl alcohol or a mixture of Nikiforov (equal amounts of alcohol and
ether), fixation time 30 min. The smear is then stained with
heating 1-2 min. Carbolic fuchsin Tsilya, washed with drinking water
and decolorize by immersing in a 2% solution of nitric acid in
alcohol or in a 1% solution of sulfuric acid, so that the preparation does not contain
it was visible traces of dye.

Explore 10 fields of vision, count the number of spores,
expressing in percent. A sufficient number is considered not less than
90% of the spores in the field of view from the total number of cells.

After completion of spore formation, test microorganisms
gently with a spatula (made of wire) or sterile
Glass beads are washed from the surface of the agar with 5-10 ml of sterile

drinking water (depending on the capacity of the vial) and pour into
containers (test tubes, vials), which are closed with sterile
rubber stoppers.

To assess the quality of the spore suspension obtained,
microorganism and making a decision about the possibility of using it
by appointment, from the vial (after thorough mixing by
shaking), 10 ml of the suspension are sterilely withdrawn into the tube and
determine compliance with requirements for biological concentration
(BC) spores of the test microorganism in the suspension and their resistance to
standard physical and chemical disinfectant and
sterilizing agents as shown in Table 5.8.

In case of contamination of the initial strain of the test microorganism
extraneous microflora is isolated by pure culture by its accepted
methods (heating, washing, centrifugation, etc.).
The isolated culture of the test microorganism is identified and
check its resistance to standard disinfectants and
sterilizing agents.

1.7.1.3 Determination of the biological concentration of the test microorganism in a spore suspension

Definition carry out method successive decantent dilutions of the suspension of the test microorganism in Sterile distilled water with subsequent suspension of the suspension in Petri dishes with a dense nutrient medium (Hottinger agar, SPA, IPA). After a certain incubation time with the appropriate temperature is the count of the grown colony forming units (CFU) and determine the number of viable spores in one ml suspension (BK).

For testing, materials and the equipment listed in Appendix 3.

During the test, the following conditions are met:

1) use variable volume dispensers of class 2 or higher accuracy;

2) in the process of performing the experiment observe aseptic conditions;

3) control the temperature of the thermostat and the incubation time crops.

Before holding testing carry out the following preparatory operations:

– melted in a boiling water bath dense nutritious medium (agar) and cool it to a temperature of 45 ± 5 ° C;

– τηε cooled nutrient medium is dispensed into 25 ± 5 cm 3 in Sterile Petri dishes in the flame of an alcohol lamp (gas burner) and Leave the cups on a horizontal surface until it freezes agar;

– if necessary, dry the plates with a dense nutritious the medium in the thermostat lids down;

– poured into sterile test tubes with cotton-gauze plugs 4.5 cm3 of sterile distilled water.

In the work, only conditioning batches of nutrient environments, for which preliminary quality is checked by sowing

reference culture of the corresponding strain. For conditioning
medium, the number of test microorganisms that have grown from their total number
should be at least 50%.

When determining the BC of the test microorganism in the initial suspension
a dispute of agar culture, the latter is bred sterile
distilled water to a concentration corresponding to
bacterial standard of turbidity of 1 billion microbial bodies in 1 ml.
Then, 0.5 ml of spore suspensions are removed by a sterile pipette and
is transferred to a tube containing 4.5 ml of sterile
distilled water. The resulting dilution (10^{-1}) carefully
shake. Similarly, by changing the pipette, all subsequent
to the necessary (10^{-6}), theoretically appropriate
concentration $1 \cdot 10^{3}$ spore in 1 ml. Of two consecutive
10-fold dilutions of the initial suspension produce seeding
0.1 ml per surface of three Petri dishes with agar (Hottinger agar,
SPA, IPA). Petri plates are incubated at a temperature of 55 ± 1 ° C or
37 ± 1 ° C depending on the type of culture for 24-48 hours, after which
determine the number of CFUs. The number of viable spores in the original
The suspensions are defined as the arithmetic mean of the CFU number c
taking into account the dilution of the initial suspension and the volume of the sample for inoculation.

An example of calculations.

Suppose that when sowing on three cups of Petri with agar
of the suspension in a 1: 100,000 dilution was calculated at 140, 110, and 134 CFU.
Similar seeding from a dilution of 1: 1 000 000 led to the formation of
12, 14 and 16 cfu.

We calculate the total number of CFUs found in all three cups
Petri of the corresponding dilutions:

1: 100,000 140 + 110 + 134 = 384

1: 1 000 000 12 + 14 + 16 = 42

The average number of CFUs on the plates will be for breeding

1: 100,000 384: 3 = 128

1: 1 000 000 42: 3 = 14

From the calculation of the inoculum (0.1 ml per cup), we calculate the number of viable spores in 1 ml of the initial suspension, taking into account dilutions, then we find the arithmetic average of the CFU number:

128 · 10 · 105 = 12.8 · 107

14 · 10 · 106 = 14,0 · 107

Thus, the number of viable spores in the initial suspension will be: (12.8 + 14.0) · 107: 2 = 13.4 · 107 = 1.32 · 108 spores / ml.

Or when sowing from one last dilution of 0.1 ml for 5 Petri dishes, calculate the concentration of viable microorganisms in 1 ml of the suspension of the drug is carried out by formula:

BC = x · p · 2,

Where:

BC - the concentration of viable spores of the test microorganism, CFU · ml;

x is the total number of colonies grown on five plates, CFU;

p - dilutions;

2 - the coefficient leading to the measurement of the sown volume suspension to 1 ml.

For example: the total number of colonies on 5 Petri dishes was 540 CFU, then the number of viable spores in the original of the suspension is: BK = 540 · 2 · 106 = 1080 · 106 = 1.08 · 109 spores / ml.

Hermetically sealed with sterile stopper vials (test tubes) with The initial suspension of spores is stored in a refrigerator at a temperature of 6 ± 2 ° C for up to 6 months if the spores of the test microorganism correspond above requirements (Table 1). To reduce the negative

effect on the spore suspension of the temperature difference,

extracting the vial from the refrigerator to select a portion of the suspension,
necessary for the study of DS, SS and their substances,

the spore suspension of the test microorganism from the vial is expedient

pack into tubes and use them as needed.

The purity of the culture of the test microorganism at all stages

cultivation is controlled by sowing it on petri dishes with

Hottinger agar, SPA, MPA.

Sibiriazvennuyu live dry vaccine (STI-1) in the study

Sporocidal activity and efficacy are used in the form of

a suspension containing 10^{8} -10^{9} spore / ml, prepared by dilution

the contents of one ampoule in 10 ml of sterile drinking water.

1.7.1.4 Methods for determining the stability of spores of test-microorganisms to reference DS

1.7.1.4.1 Determination of spore stability against a flowing steam, water Saturated steam under excess pressure, dry hot air

Resistance to the action of a fluid spore of B. cereus spores, B. subtilis,

B. anthracis, anthrax vaccine STI-1 is determined in the apparatus

Oyl-Mueller, using cambric test objects,

contaminated with the above test microorganisms, with

the subsequent sowing of test objects in a liquid nutrient medium.

The Oyl-Muller apparatus can be replaced by a device,

available for manufacturing in almost any laboratory, where

it is necessary to conduct such a study. For this, the glass

a 1-2 l flask with a wide elongated throat. Cork stopper

under it cut off on 1/3 on length for maintenance of an exit of steam. it

The hole is also used for thermometer measurements

Steam temperature at the location of the batist test objects. Across

A wire passing through the end of the welded

(or reinforced in another way) perpendicular to it

metal mesh with a diameter of 3-3.5 cm in stainless steel,

intended for placing cambric test objects,

contaminated with test-microorganism spores.

Wire in a stopper is installed so that the reticulum is located
In the place where the cone of the bulb passes into the throat. This ensures the passage of
through a mesh of almost the entire volume of emitted boiling in a flask
water vapor. The water level should be from the net at a distance of
5-6 cm.

During the test, the following indicators are monitored:

temperature of the fluid vapor;

initial and residual contamination by test microorganisms
batistovyh test objects;

The time of action of steam for contaminated test objects in
apparatus Oyl-Muller (flask).

The tests are carried out as follows: poured into a flask
distilled water and heated to boiling. When
temperature of 100 ° C on a thermometer under the influence of
fluid couple, on in advance sterilized
autoclaving together with a cork or burned in a flame
The grid is placed 2 batistovyh test object (1 × 0.5 cm),
contaminated disputes test-microorganism (methodology
for the preparation of test objects, see 5.1.2.2.). Test objects place
so that their contact with the wall of the neck of the bulb is excluded upon introduction
Setochki in the flask. Holding on to the cork, a grid with test objects is added to the
zone of action of the fluid vapor and include a stopwatch. At the end of

2 minutes. effects of steam, holding on to the plug, mesh with test objects
are removed from the flask, and the test objects are immediately placed (sown) in two
test tubes with sterile nutrient broth. Burn the mesh and
put on it 2 new test objects. Similarly to the above
bring them into the area of steam for 2 minutes, then they also extract and
are placed in test tubes with sterile nutrient broth. So
The operation is repeated, increasing the exposure by 1 min. up to 10 min.
The cultures are incubated at 37 ± 1 ° C for 7 days. Preliminary
the results are recorded after 48 hours of incubation,
the final - on the 7th day. From test tubes with sprouted broth
plant a loop on a solid medium to identify the grown
test microorganisms.

When using the Oill-Müller apparatus, it is not necessary allow accidental contact with the neck of the bulb first to disinfection of test objects, then - after disinfection. These errors that distort the results of the experiment can be avoided when using the Oyl-Muller apparatus in the modification of LA Blinova (Figure 2). There are four openings in the fluid tank. The first hole (1) is placed at the top and is intended for a thermometer, The second (2) - on the right in the end - for entering infected test objects, the third (3) - on the front side - to remove the disinfected test-objects and the fourth (4) - left at the end - to exit the steam. When using such a device, test objects are punctured on the needle, fixed in a plug, which is inserted into the hole 2. Hole 3 during exposure is covered with a sterile stopper. At the end Exposure is opened and through it is extracted by burned tweezers taken from the needle test objects, which are immediately sown in bouillon.

The thermometer used in the device must have a scale with divisions by tenths of a degree. Determination of stability bacilli to a flowing vapor is carried out at atmospheric pressure close to 760 mm Hg. Art. Accounting for this factor is important, since it can significantly influence the results of the evaluation. Thus, at atmospheric pressure 745 mm Hg. Art. the temperature of the fluid vapor is 99.4 ° C and resistance at this temperature B. cereus is 6-7 minutes, and when pressure of 765 mm. gt; Art. the temperature of the steam is 100.8 ° C, the resistance is not exceeds 3-4 minutes.

Spores of test microorganisms should be resistant to flowing steam temperature of 100 ° C for at least 7-9 minutes.

Stability of the test microorganism G. stearothermophilius to water saturated vapor under excessive pressure. As test-carrier used bottles of neutral glass, contaminated suspension dispute test-microorganism G. stearothermophilus.

Pre-vials are thoroughly washed and sterilized

steam or air. From the initial suspension, the spores are prepared
Working suspension for contamination of test carriers.

Sterile test carriers are contaminated at the rate of (1-5) · 10_6
test-microorganism G. stearothermophilus, which is achieved by introducing into
each carrier with a variable volume dispenser of 0.02 ml
spore suspensions with a content of $5 \times 10_7$ to $2.5 \times 10_8$ spores / ml.

Figure 1.1. Schematic diagram of the device device
Oyl-Mueller in the modification of LA Blinova.

The contaminated test carriers are dried in a thermostat at
temperature 37 ± 1 ° C for 24 hours and put into paper
packages approved for use as sterilization
packaging materials in the Russian Federation.

Stability of spores of the test-microorganism G. stearothermophilius
to the water saturated vapor under excess pressure is determined
in a steam sterilizer with a volume of 75 dm_3 with a gravitational method
preliminary removal of air from the sterilization chamber.

Packaged test carriers are placed in a sterilization box into the unloaded chamber of the steam sterilizer. After dialing The pressures in a water vapor chamber of 1.1 ± 0.1 kgf / cm 2 are displaced air from the steam sterilizer chamber (steam purging sterilizer) for 10 minutes. (with an open drain cock and

pressure in the sterilization chamber from 0.1 to 0.2 kgf / cm 2). After purge the steam pressure in the sterilization chamber up to 1.1 ± 0.1 kgf / cm 2 , at 121 ± 1 ° C and after 5 min. (survival time spore test-microorganism) from the moment the pressure is established, steam is released. For reducing the exposure time of steam before and after the test period exposure (exposure time at a temperature of 121 ± 1 ° C rise pressures are held for a maximum of 8 minutes, and descent for 3 minutes. The temperature is controlled by the maximum thermometers (SP-82).

A similar study is conducted with a 15-minute time influence (the time of death of the test-microorganism spore). For each of the of the periods impacts exhibited not less 10 test carriers. At the end of the exposure time, test media remove from the sterilizer. In the vials, 1 ml of nutritious medium (Hottingering broth, BCH, SPB, color medium with bromocresol purple indicator), close with sterile rubber plugs (N7.5) and incubated at a temperature of 55 ± 1 ° C for 7 days with the use of nutrient broth (broth Hottinger, SPB, BCH) or within 48 hours of use color medium with bromocresol indicator purple.

The results are recorded by visual inspection. No opacification / discoloration of culture medium with the indicator indicates the death of the dispute. In the presence of growth microorganisms conduct comparison the last from test-microorganism.

As a control, test media are used which are not were subjected to the action of a sterilant. Crops of control

test media and nutrient media, as well as incubation of crops analogous to the experimental test carriers that were subjected to the action of a sterilant.

The time of death of spores of G. stearothermophilius under the action of water saturated steam at an excess pressure of 1.1 ± 0.1 kgf / cm ^{2} , The temperature of 121 ± 1 ° C should be at least 15 minutes.

Stability of B. licheniformis spores to dry hot air is determined in an air sterilizer of 80 dm ^{3} volume from forced circulation and air speed more than 1 m / s, which provide permissible limit deviations of the nominal temperature.

As a test carrier, bottles of neutral glass, contaminated disputes test-microorganism B. licheniformis. Pre-flasks are thoroughly washed and sterilized by steam or air. From the initial suspension a working suspension is prepared for the contamination of test carriers from calculation (1-5) × 10 ^{6} spores in the carrier.

Sterile test carriers are contaminated with a working suspension The test-microorganism dispute of B. licheniformis, which is achieved by introducing into each test vehicle with a variable volume dispenser 0.02 ml of spore suspension in distilled water with a content of from 5.0 × 10 ^{7} to 2.5 × 10 ^{8} spores / ml.

The contaminated test carriers are dried in a thermostat at temperature 37 ± 1 ° C for 24 hours and put into paper or polymer bags approved for use as sterilization packaging materials in the Russian Federation.

Packaged test carriers are placed on a shelf of air sterilizer preheated to 140 ° C. Sterilizer

close and after reaching a temperature of 160 ± 3 ° C, the countdown begins aging time. After 4 minutes. (survival time spore test-

microorganism) the device is switched off. Temperature control

carried out by an external thermometer.

A similar study is conducted with a 30-minute time

the test effect (time of death of the test-microbe spores).

At each of the indicated periods of the test exposure

exhibit at least 10 test carriers.

At the end of the exposure time, the test media is removed from the

sterilizer. In the vials, 1 ml of the nutrient medium (broth

Hottinger, BCH, SPB, color nutrient medium with an indicator

bromotymol blue) and cover with sterile rubber

plugs (N7.5) and incubated at 37 ± 1 ° C for

7 days with the use of nutrient broth (Hottinger broth,

SPB, BCH) or within 48 hours using a color

a medium with a bromothymol blue indicator.

The account of the results of the experiments and control is carried out analogously as in

determination of the stability of the spores to a saturated water vapor under

pressure.

Time of death of spores of test-microorganism B. licheniformis at

dry hot air at a temperature of 160 ± 3 ° C

be at least 30 minutes.

1.7.1.4.2 Determination of spore resistance to chloramine, peroxide hydrogen, glutaraldehyde

Resistance to chloramine, hydrogen peroxide, glutar

aldehyde in B. cereus spores, B. subtilis, B. anthracis, live dry

anthrax vaccine STI-1 is determined by immersion

batistovyh test objects, contaminated with a spore suspension

of these cultures (5.1.2.2) in a 10% chloramine solution, 6% solution

hydrogen peroxide, 2.5% solution of glutaraldehyde followed by

neutralization of active substances and planting in liquid

a nutrient medium.

AT process tests control concentration

active substance in the working solution of DS, its temperature in the experiment, the initial and residual level of contamination by spores test-microorganism of the test object, the time of exposure of test objects in tested disinfectant solution.

Determination of spore resistance to chloramine.

Iodometric method determines the percentage of active chlorine in chloramine. In the experiments, a preparation containing 26-28% active chlorine, dissolving which in water is prepared 10% (by the preparation) working solution. Prepare and pour into tubes of 5 ml sterile neutralizer solution (2% solution of sodium thiosulfate), sterile drinking water, nutrient broth (MPB). Prepared and contaminated test-microorganism, batistovye test objects (see clause 5.1.2.2.). If in use previously prepared and stored in refrigerator contaminated with test-microorganism spores test objects, they are preliminarily removed from the refrigerator so that they have acquired room temperature (18-20 ° C).

When carrying out experiments in a glass container with a volume 50-100 ml with a pipette pour the required volume of 10% solution chloramine, at a rate of 0.5 ml for each test object and placed in A water bath with a temperature of 20 ° C for the entire period of the experiment. Count in a Petri dish, the amount of contaminated spores of test-microorganisms of bath test test objects (2 per

each exposure), grab them with sterile tweezers all at once and lowered into a container with a solution of chloramine; light wiggle capacity to achieve their full wetting. At the moment of wetting all test objects mark time.

After every hour, two test objects are removed by a sterile loop from a solution of chloramine and lower them into a test tube with 5 ml of 2% sterile solution of sodium thiosulfate to neutralize the residual action chloramine. After 5-10 minutes. test objects are transferred to the second test tube with 5 ml of sterile drinking water, and after 10-15 minutes. each The test object is placed in 5 ml of nutrient broth.

To control two contaminated test-

The microorganism of the test object is immersed in sterile drinking water (instead of chloramine solution) for the maximum exposure time, then (as well as experimental test objects) they are transferred successively to solution neutralizer (sodium thiosulfate), sterile drinking water and sow in a liquid nutrient broth in test tubes. Completeness of neutralization active chlorine control by immersions non-contaminated test objects in a 10% chloramine solution maximum exposure, then into a solution of sodium thiosulfate, washed in water and placed in broth, where 0.1 ml of the suspension is added, containing 20-30 viable test-microbe spores. Growth culture in the broth indicates effective neutralization effects of chloramine.

Experimental and control sowings are placed in a thermostat at temperature 37 ± 1 ° C; The presence of growth of the test microorganism is checked in 48 hours. From test tubes with growth, a loop is made on a solid nutrient medium for identification of test microorganisms.
The final recording of the results is carried out after 7 days.

The experiment is repeated at least 3 times. Spores of test microorganisms: B. cereus, B. Subtilis, B. anthracis, live anthrax vaccine STI-1 should be resistant to a 10% solution of chloramine for at least 360 minutes.

Determination of the stability of spores to hydrogen peroxide.

Iodometric method is used to determine the concentration of peroxide hydrogen in the medicine "Peroxide of hydrogen". In the experiments use a vehicle containing at least 30% hydrogen peroxide, from which by dilution with sterile drinking water a solution is prepared for studies containing 6% hydrogen peroxide. For neutralization of hydrogen peroxide, a sterile 2.5% solution of sodium thiosulfate. Preparation and definition of sustainability The test-microorganism spores to a 6% hydrogen peroxide solution by the same procedure as to chloramine, only as a The neutralizer used 2.5% sodium thiosulfate solution.
Given that the spores of test microorganisms must be resistant to 6% hydrogen peroxide solution for at least

60 minutes, the test is carried out for 90 minutes. with sampling
(2 tests each) every 15 minutes. (usually take 2 test objects per 6
expositions). Similar, as in the determination of the stability of the spores to
chloramine, sowing, recording results and monitoring.

Spores of test microorganisms: B. cereus, B. subtilis, B. anthracis,
live anthrax vaccine STI-1 should be resistant to 6%
solution of hydrogen peroxide for at least 60 minutes.

Determination of the stability of B. subtilis spores to a 2.5% solution
glutaraldehyde.

Determine the concentration of glutaraldehyde in the original
(concentrated) solution of glutaraldehyde. In experiments
use a preparation containing at least 20% glutaraldehyde.

A test solution containing 2.5% glutaraldehyde,
are prepared by diluting the original solution with sterile drinking
water, followed by adjusting the pH of the prepared solution to
of the values of 7.5. Prepare and determine the stability of B. subtilis spores to
2.5% solution of glutaraldehyde is carried out by the same procedure,
as well as to chloramine, using batistovye test objects,
contaminated with this test microorganism, only as a
neutralizer use either a sterile 1% bisulfite solution
sodium or a sterile universal neutralizer containing
Tween 80 (3%), saponin (0.1%), histidine (0.1%), cysteine (0.1%).
Given that the spores of some test-microorganisms must be
are resistant to 2.5% glutaraldehyde solution
less than 3 hours, the test is carried out for 6 hours
with sampling (2 test objects) every 30 minutes. Crops, accounting
results and control is performed in a similar way
determination of spore resistance to chloramine.

Spores of the B. subtilis test microorganism should be resistant to
2.5% glutaraldehyde solution for at least 3 hours.

1.7.2 Ensuring the standard of the research environment
sporicidal activity of DS, CC and their substances

Chemical-analytical control of DS, SS and their substances. Before

research of sporicidal activity of DS, SS and their
substances is necessary analyze submitted by
approved formulations and technical means
conditions for domestic or specification for foreign funds,
carry out chemical-analytical studies by definition
concentration of active substances and determine the conformity of it and

other indicators, regulated the above
documents. At the same time, chemical analytical methods are used
control and apply storage conditions and safety measures
when working with it, the means proposed by the manufacturer.

The choice, cooking and control effectiveness of
neutralizers of DS to exclude residual sporicidal
or sporostatic effect of DS, MOP and their substances on the test-
microorganisms. For disinfection and sterilization apply
means having a sporicidal effect, that is, killing
Spores, but do not delay only their growth. Therefore, in determining
sporicidal action, it is necessary to distinguish between sporicidal
action of the drug against sporostatic.

Based on the accumulated experience to neutralize
antimicrobial action of DV from various chemical groups
(depending on the concentration of DV in the solution) the following
neutralizers:

for means of groups oxidants (chlorine-, iodine-,
peroxide-containing products; preparations containing per peraceous
acid, ozone) - 0.5-2.5% solutions of sodium thiosulfate;

for aldehyde and phenol-containing products - universal
a neutralizer containing 3% polysorbate 80% (Tween 80), 3%
saponin, 0.1% histidine, and 0.1% cysteine; or 3% polysorbate 80%,
2% histidine, 0.3% lecithin, 3% saponin;

for composite DS - universal neutralizer,
for example, containing 3% polysorbate 80%, 3% saponin, 0.1%
histidine and 0.1% cysteine or 3% polysorbate 80%, 3% saponin,
0.3% lecithin and 0.15% cysteine, 0.15% sodium thiosulfate, or other

neutralizers recommended by manufacturers.

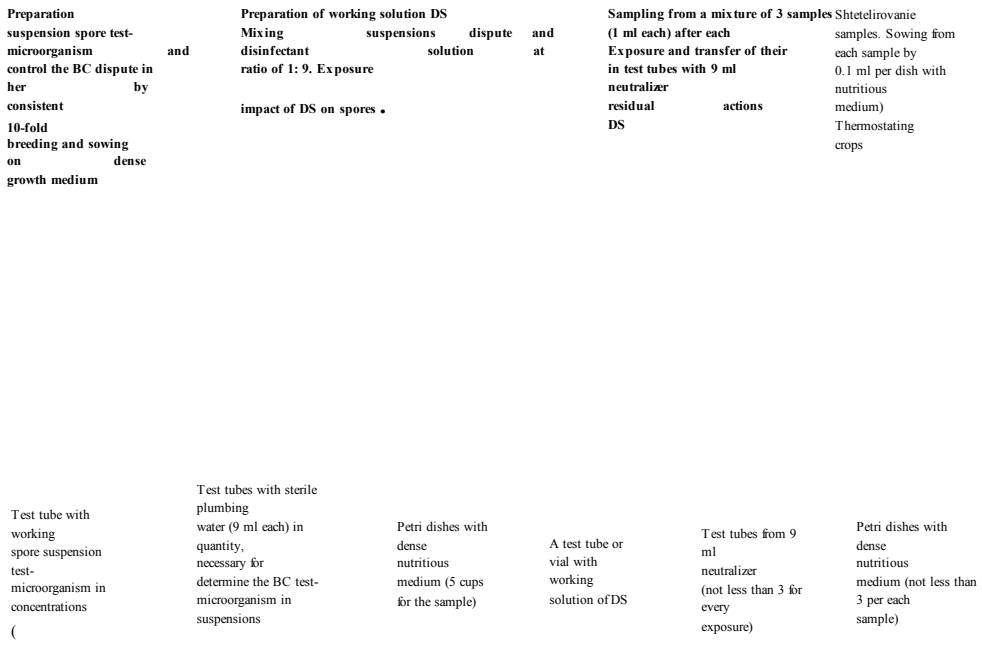
Solutions of neutralizers are prepared in aseptic conditions, using only sterile distilled water. When impossibility of observing aseptic conditions of preparation neutralizers, sterilization of ready solutions autoclaving at 1.1 kG / cm 2 (121 ° C) for 15 minutes. The temperature of neutralizer solutions should be plus 20 ° C, regardless of the ambient temperature. Finished solutions must be used on the day of preparation. Storage is allowed ready solutions at 4 ° C for 48 hours.

Control of completeness of neutralization of residual action tested means.

To monitor the effectiveness of the neutralizer and completeness neutralization of the residual effect of DS are used suspension method, which provides the most stringent conditions for action neutralizer in the tests of DS. The sequence and methodology for performing basic operations in carrying out such experiments and their purpose are shown in the diagram of Fig. 5.2. and in Table 5.10.

Security techniques security at research sporicidal activity of DS, CC and their substances. Disputes of the test-microorganisms are highly resistant, so their death in most cases, high concentrations of DS, SS and their substances for both the preparation and for DV. Therefore, when stored, weighing, preparation of working solutions, their chemical-analytical studies and experiments apply the protection measures provided for in the technical specifications for domestic funds or specifications - to foreign ones, taking into account class of their danger.

Fig. 1.3 Diagram of the algorithm for carrying out the experiment on the evaluation of sporicidal activity of DS suspension method



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In experiments with the virulent test-microorganism of the pathogen anthrax, except for sowing on a solid nutrient medium, a sample 0.2 ml are administered intraperitoneally to white mice weighing 10-12 g. In parallel, the animals are subjected to a nutrient challenge medium with a neutralizer and spore suspension of the original test-microorganism. The number of animals in the control groups - not less than 3. Observe the animals for 48-96 hours.

Both dead and euthanized mice are opened, do sowing organs on elective media to detect growth of the pathogen anthrax and its identification; incubating Petri dishes with samples at a temperature of 37 ± 1 ° C or 55 ± 1 ° C, depending on the test microorganism for 2-7 days and recording the results.

Effective exposure for working solution tested

concentration is considered the second exposure of the lack of viable spores in the crops of their respective samples.

The number and interval (step) of exposures at which carry out sampling for the effectiveness of DS, are selected on the basis of accounting data on the composition and effectiveness of those entering the facility active substances.

The agent, solutions of which are provided at room temperature temperature for 60 min. complete loss of the dispute of one of the recommended spore test microorganisms are considered as a promising sporicidal agent for further study: determination of the spectrum of sporicidal action, factors affecting sporicidal activity of DS, etc.

1.7.3.2. The method of cambric test objects

The method of the cambric test objects is used to obtain information on the concentration and time of an effective sporicidal activity DS. In principle, the methodology of implementation an experiment to assess the sporicidal activity of DS method sampler test objects is shown in the diagram of Fig. 5.4.

As can be seen from the diagram in Figure 5.4, Sporocidal activity of DS by the method of bathist test objects must be prepared:

Sterile batistovye test objects measuring 1x0.5 cm by dipping a piece of cambric for 24 hours in cold drinking water for remove the appliqué. Then the fabric is carefully washed with soap, rinsed in cold water, boiled, dried and patted hot an iron. With the help of a needle in a prepared piece of tissue, thread in the longitudinal direction at a distance of 11 mm from each other, and in the transverse direction is 6 mm. On these lines the batiste is cut scissors for individual test objects; lay out 50 pieces in each Petri dishes, packed in paper and sterilized in steam a sterilizer;

Working suspension of test microorganism with concentration
not less than 1 · 10 9 spores / ml for contamination of cambric test objects;

test tubes with sterile drinking water (10 ml) for monitoring
the actual biological concentration (BK) of the test microorganism at
contaminated test objects;

Test solution of DS in the vessel (test tube, flask or beaker)
in an amount sufficient to soak all the test-
objects, based on calculation of 0.5 ml of solution per 1 test object;

required number of tubes (depending on the amount
Samples taken to determine the time of complete spoilage of the test-
microorganism under the action of DS) containing 9 ml
neutralizer, previously tested for efficiency
neutralization of the residual effect of the tested DS on
test microorganism (see 5.8.2.);

Petri dishes with sterile dense nutrient medium for
sowing control samples of the test-microbe of the test-microbe at the test facility and samples
control the effectiveness of the action of DS on the test microorganism.

The methodology for carrying out the actual experience in the evaluation of sporicidal
activity of the DS provides, as can be seen from the scheme, a consistent
Perform the following steps (Figure 5.4):

careful mixing of stored in vitro or in vial
at a temperature of 6 ± 2 ° C of the initial suspension of the agar culture test-
microorganism by shaking the vial (vial) for
2-3 minutes; preparation of the working suspension of the test microorganism
in a concentration of not less than 1 · 10 9 spores / ml and in an amount sufficient for
contamination of the test objects used in the test (at a rate of 0.2 ml
on the test object);

contamination of the batist test-objects by a microorganism test.
To do this, place in the Petri dish the amount required for the experiment
sterile batistovyh test objects; pour them cooked
working suspension of the test-microorganism spores, providing them with
uniform wetting, and leave them in suspension in a closed cup
Petri for 20 min. With observance of aseptic contaminated test-

Objects are transferred to sterile filter paper laid
in two layers in a sterile Petri dish, cover them with a sheet of the same
paper and cover the cup with a lid; in 10 minutes. shift

test objects on dry sterile filter paper in
sterile Petri dish and dried in a thermostat at 37 ± 1 ° C in
flow 60 min. with a slightly open lid. Dried up
test objects, contaminated with test-microorganism spores,
is placed in Petri dishes in a refrigerator and stored at a temperature of
6 ± 2 ° C, using for experiments within no more than 3-4 days;

for the purpose of controlling microbial contamination of test objects
determine the BC dispute on them. To do this, contaminated with spores
batistovye tests are immersed in test tubes with 10 ml of sterile drinking
water and shaken for 10-15 minutes. on the laundering shaker
dispute with the test object. Then make 3 consecutive 10-fold
dilution to obtain a suspension with a concentration of the order of 1×10^3
spores / ml, from which 0.1 ml of culture is produced for 5 cups with a dense
nutrient medium. The cultures are incubated in a thermostat at 37 ± 1 ° C or
55 ± 1 ° C for 2-4 days, and the total amount
grown on the cups of CFU. Using the BC definition formula,
5.8.1.3., calculate the number of viable
the test-microbe on the test object;

preparation of the test solution of DS in
corresponding capacity at the rate of 0.5-1.0 ml per test facility;

The room of the tested working solution DS for water
bath with a preset temperature. If the task of the experiment is not
The influence of temperature on
effectiveness of the tool, then the evaluation of the effectiveness of the solution
The test substance is carried out at a temperature of 18-20 ° C;

soaking necessary to ensure sampling
the quantity of contaminated test objects in the disinfectant

solution with a preset temperature and a countdown to the stopwatch of time the initiation of exposure of the DS to the test microorganism;

selection (at the end of each preset exposure) with a sterile tweezers (loops) of test objects (at least 3 per exposure), which is placed in a test tube with 10 ml of previously tested for efficiency neutralizer of the residual effect of DS on the test microorganism.

Determination of the presence of viable spores on the test object. If Only the establishment of the availability for this Exposure of viable controversies remaining on the test object test-microorganism, then the sample is not shaken, and the tests Sterile tweezers are removed from the neutralizer and placed in a vial with sterile nutritious broth. If envisaged quantitative determination of remaining test-object viable spores of the test-microorganism, then Sterile dense nutrient medium in Petri dishes is sieved in 0.1 ml sterile pipette from a neutralized sample (or from of dilution) obtained after intensive shaking test objects manually for 5-10 min. on the shaker;

Fig. 1.4 Scheme of the algorithm for carrying out the experiment on the evaluation of sporicidal activity of DS by the method of ca test objects.

Preparation and contamination sterile test objects	**Dough drying on the filter. paper and 20 min in an open cup Petri at Thermostat. Test selection in 10 ml d.v. for holding control the original contamination by appropriate (before 10_3) sample dilution and sowing on dense medium of 0.1 ml.**	**Soak the tests (3 test exposition) on at disinfectant solution**	**Sampling of prototypes (3 each test) after each exposition in test tubes with 10 ml neutralizer residual actions DS**		Sowing test for 3 cups with nutritious medium (0.1 ml each neutralizer after shaking- test tube), dough room in the broth.
Petri dish with spore suspension test-microorganism (	Test tubes with sterile-distilled water (10 ml each) in quantity, necessary for determine the BC test-microorganism on test	Petri dishes with dense nutritious medium (5 cups for the sample)	Capacity with disinfect solution (from the calculation 0.5 ml per 1 test)	Test tubes with neutralizer m (9 ml each)	Test tubes with nutritious broth (not less than 3 per exposure)

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Incubation of crops in Petri dishes or test tubes is carried out at a temperature of 37 ± 1 ° C or 55 ± 1 ° C depending on the type of test-microorganism for 2-4 days;

accounting and analysis of the results of the experiment (test).

Effective exposure for working solution tested concentration is considered the second exposure of the lack of viable spores in the crops of their respective samples.

The number and interval (step) of exposures at which sampling is carried out for the effectiveness of the DS, data on the composition and effectiveness of the substances.

The agent, solutions of which are provided at room temperature temperature for 60 min. complete loss of the dispute of one of the recommended spore test microorganisms, can be considered as a promising sporicidal DS for further study.

Study of the spectrum of sporicidal activity of chemical DS, MOP and their substances are carried out using the following test-

microorganisms: B. cereus, B. subtilis, B. anthracis, anthrax
dry live vaccine STI-1. Further studies are carried out,
using the most stable test microorganism.

1.7.3.3 Methods for studying factors affecting the sporicidal activity of DS, SS and their substances

Spectrum of sporicidal activity of DS, CC and their substances and its dependence on temperature, pH value and the presence of protein pollution.

Studies are carried out using the method of bathist test objects (p.5.8.3.2.).

Determination of the spectrum of sporicidal action of DS, SS and their substances use spore test microorganisms, Recommended for the study and evaluation of sporicidal activity (see 1.8.1.1., Table 1.8.).

Based on the obtained data, the feasibility is determined further studies for use as DS, SS and their substances.

Determination of the effect of temperature on sporicidal activity The DS, SS and their substances are conducted in order to identify the possibility use of heated DS solutions to shorten the time disinfection of objects with respect to the test-microbial spores, and also to assess the effectiveness of the sporicidal action low ambient temperatures, disinfected object and the DS solution itself.

To study the effect of temperature, working solutions subjects DS are prepared on the day of the experiment, poured into glass flasks (test tubes) at a rate of 0.5 ml for each test object.

Investigation of the influence of positive temperatures of the DS solution on its sporicidal activity is carried out using a water bath, in which a container with a disinfectant solution is heated up to 18 ± 1 ° C, 37 ± 1 ° C, 55 ± 1 ° C, and then immersed in it

Contaminated test objects and maintain these temperatures in the process of the whole experience.

Experiments to assess the effect of reduced temperature on the activity of the DS is carried out using a cryostat or saline low-freezing solutions in which the container is cooled with

disinfectant solution up to 10 ± 1 ° C, 5 ± 1 ° C, minus 20 ± 1 ° C and keeping them in the process of experience. After reaching the temperature in a solution of DS submerged batistovye test objects, contaminated with a test microorganism from the calculation of 2 test objects for each exposure.

At regular intervals from each flask 2 test-subjects are removed and placed in test tubes with appropriate neutralizer for 5 minutes, then the second test tube with sterile drinking water for 5 min. and only after this each test object is transferred to a tube filled with 5 ml of broth Hottinger (pH 7.2). The crops are incubated for 48-72 hours at 37 ± 1 ° C. Control serves by 2 test objects at each investigated temperature, not subjected to the action of the subject DS, but immersed in test tubes with sterile drinking water for a period of, equal to the action of the subject DS.

Determination of the effect of pH on sporicidal activity DS, SS and their substances begin with the preparation of working solutions DS, having a different pH value (5.6-6.0, 7.0, 8.5-9.0). For Acidification of the solution using decinormal solution of hydrochloric acid or other acid, and for alkalization - decinormal solution alkali. Prepared solutions immerse the contaminated spores batistovye test objects. The study of dependence sporicidal activity of DS, CC and their substances from the pH value are carried out according to the procedure described above, only when neutralizing The actions of active substances simultaneously reduce the value of pH, adding acid or alkali, respectively.

Determination of the effect of protein contamination on the sporicidal activity DS is conducted in order to identify the possibility of influence (or

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of establishing him absence) protein pollution on disinfected object for the sporicidal activity of the DS.

The study is carried out using the method of cambric test objects (paragraph 5.8.3.2.), only for the contamination of test objects are used slurry dispute test-microorganism, containing 20% inactivated serum of bovine animals or defibrinated blood, which is added to the suspension at its cooking. Inactivation of normal bovine serum The cattle are carried out by a fractional three-fold heating in a water bath at a temperature of 60 ± 1 ° C for 30 minutes. If the activity of the drug does not decrease in the presence of 20% protein, the concentration inactivated serum of bovine animals or defibrinated blood in the suspension of the test microorganism, increase to 40%. No decrease in sporicidal activity DS at addition and 40% of serum allows to consider DS not reacting to the presence of protein contamination.

1.7.4 Methods for studying the sporicidal efficacy of DS, intended for disinfection of objects of the external environment, contaminated test microorganisms in a spore form

List of test objects modeling objects subject to disinfection, includes: surfaces of premises, furniture, apparatus, devices, sanitary equipment, transport means, etc .; medical products, including endoscopes; subjects of nursing, toys; tableware, laboratory and from under the discharge; Linen, clothes, overalls and other objects from the tissues; rubber products, including gloves, boots, aprons, etc .; footwear; Hands in rubber gloves; remnants of food;

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allocation: feces, urine, blood, sputum; water; air; medical

waste.

1.7.4.1 Study of sporicidal effectiveness of DS, intended for disinfection of surfaces of premises, furniture, apparatus, instruments, sanitary equipment, vehicles and other objects

In the studies, test surfaces (10 × 10 cm) from various materials: wood (painted with oil or glue and other paints; covered with wallpaper and unpainted), linoleum, plastic, tiles, faience, tiles, metals, glass.

Test-surfaces of various materials (except for surfaces painted with glue paint and pasted wallpaper) thoroughly washed with water and soap and a brush, sterilized in a steam sterilizer. Test surfaces, painted with glue and wallpapered, wallpapered several times with a sterile gauze a napkin moistened with sterile drinking water. Prepare a spore suspension of the test microorganism (B. cereus, anthrax living dry STI-1 vaccine for humans, B. anthracis) containing $2.0 \cdot 10^9$ spores / ml, 40% horse serum inactivated at 56 ° C. in within 30 minutes. (to 6 ml of a suspension containing 2.0×10^9 spores / ml, add 4 ml of serum).

After drying, the test surface is positioned horizontally and on them using a single-channel mechanical dispenser or glass pipettes cause 0.5 ml suspensions dispute test-microorganism. The suspension is evenly distributed throughout the test-surface (100 cm^2) with a sterile glass spatula. If the suspension of the test microorganism is not evenly distributed, and is collected in a drop, grinding with a spatula on the test surface

are carried out repeatedly (3-5 times). Contaminated test-surfaces are dried at room temperature until complete drying (30-120 minutes). Disinfection of test surfaces are carried out by methods of wiping or irrigation with disinfectant solution (when working with B. anthracis, only irrigation in the biological protection box is not pre-dried

more than 20 minutes).

In the behavior of experiments, the test surfaces, colored glue and other paints, or pasted wallpaper, have vertically and disinfected by irrigation with disinfectant solution. The remaining test surfaces are disinfected as in horizontal, and in vertical positions by irrigation, single-entry or twofold wiping, or washing disinfecting solution.

Depending on the type of surface to be treated and availability contamination on it, the rate of consumption of DS by wiping is 100-150 ml / m ^{2} (1-1.5 ml per 100 cm ^{2}); method of irrigation - 150 ml / m^2 (1.5 ml per 100 cm^2) when treated with a Kvazar spray or its analogues and 300-500 ml / m^2 (3-5 ml per 100 cm ^{2}) when processing a sprayer of the "AutoMax" type or a hydro-switch.

If necessary, rubbing or Irrigation is repeated after 15 minutes.

For control effectiveness of disinfection across certain time intervals (15-30-60 minutes) from the test surfaces Do rinses by thoroughly rubbing the test surface first in one, and then perpendicular to the direction of sterile gauze cloth (5 × 5 cm), moistened with a neutralizer. After Wipe on the test surface should not remain excessive moisture.

Napkins are immersed for 5 minutes. in test tubes (containers) with a neutralizer (10 ml) corresponding to the DS under test, and then into sterile drinking water with beads and shake it on a shaker within 10 minutes. The resulting flushing liquid is added to 0.1 ml per the surface of the nutrient agar of two Petri dishes, carefully spreading over the entire surface. The crops are incubated in a thermostat at temperature 37 ± 1 ° C for 48-72 hours.

AT control experiments for processing similarly contaminated test surfaces instead of the solution of DS are used Sterile drinking water from the same calculation as the experimental ones. A liquid into which a sterile gauze pad is placed after

take the flush from the control surfaces, before planting, dilute in 100 times and 0.1 ml are added to the surface of the nutrient agar of two Petri dishes. The cultures are incubated in a thermostat at a temperature of 37 ± 1 ° C. The results are taken into account after 48 hours (preliminary), and the final - after 21 days.

Evaluation of the results of control experiments is carried out on the sowing of A dilution in which the number of CFUs on the surface of the nutrient agar in The Petri dish is from 30 to 300.

After standing in the thermostat, the number CFU on agar plates, the density of contamination is calculated per 100 cm 2 and calculate the effectiveness of decontamination, taking the number of test microorganisms taken from the control test-objects, for 100%.

For example: on crops with 100 cm 2 of the control test surface found 148,000 CFU, and with a similar type of experimental test-surface - 20 cfu.

148,000 - 100%

20s

x = 20 x 100: 148,000 = 2: 148 = 0.013%

Incubation of crops in Petri dishes or test tubes is carried out at a temperature of 37 ± 1 ° C or 55 ± 1 ° C depending on the type of test-microorganism for 2-4 days;

accounting and analysis of the results of the experiment (test).

Effective exposure for working solution tested concentration is considered the second exposure of the lack of viable spores in the crops of their respective samples.

The number and interval (step) of exposures at which sampling is carried out for the effectiveness of the DS, data on the composition and effectiveness of the substances.

The agent, solutions of which are provided at room temperature temperature for 60 min. complete loss of the dispute of one of the recommended spore test microorganisms, can be considered as a promising sporicidal DS for further

study.

Study of the spectrum of sporicidal activity of chemical DS, MOP and their substances are carried out using the following test-microorganisms: B. cereus, B. subtilis, B. anthracis, anthrax dry live vaccine STI-1. Further studies are carried out, using the most stable test microorganism.

1.7.3.3 Methods for studying factors affecting the sporicidal activity of DS, SS and their substances

Spectrum of sporicidal activity of DS, CC and their substances and its dependence on temperature, pH value and the presence of protein pollution.

Studies are carried out using the method of bathist test objects (p.5.8.3.2.).

Determination of the spectrum of sporicidal action of DS, SS and their substances use spore test microorganisms, Recommended for the study and evaluation of sporicidal activity (see 1.8.1.1., Table 1.8.).

Based on the obtained data, the feasibility is determined further studies for use as DS, SS and their substances.

Determination of the effect of temperature on sporicidal activity The DS, SS and their substances are conducted in order to identify the possibility use of heated DS solutions to shorten the time disinfection of objects with respect to the test-microbial spores, and also to assess the effectiveness of the sporicidal action low ambient temperatures, disinfected object and the DS solution itself.

To study the effect of temperature, working solutions

subjects DS are prepared on the day of the experiment, poured into glass flasks (test tubes) at a rate of 0.5 ml for each test object.

Investigation of the influence of positive temperatures of the DS solution on its sporicidal activity is carried out using a water bath, in which a container with a disinfectant solution is heated up to 18 ± 1 ° C, 37 ± 1 ° C, 55 ± 1 ° C, and then immersed in it

Contaminated test objects and maintain these temperatures in the process of the whole experience.

Experiments to assess the effect of reduced temperature on the activity of the DS is carried out using a cryostat or saline low-freezing solutions in which the container is cooled with disinfectant solution up to 10 ± 1 ° C, 5 ± 1 ° C, minus 20 ± 1 ° C and keeping them in the process of experience. After reaching the temperature in a solution of DS submerged batistovye test objects, contaminated with a test microorganism from the calculation of 2 test objects for each exposure.

At regular intervals from each flask 2 test-subjects are removed and placed in test tubes with appropriate neutralizer for 5 minutes, then the second test tube with sterile drinking water for 5 min. and only after this each test object is transferred to a tube filled with 5 ml of broth Hottinger (pH 7.2). The crops are incubated for 48-72 hours at 37 ± 1 ° C. Control serves by 2 test objects at each investigated temperature, not subjected to the action of the subject DS, but immersed in test tubes with sterile drinking water for a period of, equal to the action of the subject DS.

Determination of the effect of pH on sporicidal activity DS, SS and their substances begin with the preparation of working solutions DS, having a different pH value (5.6-6.0, 7.0, 8.5-9.0). For Acidification of the solution using decinormal solution of hydrochloric acid or other acid, and for alkalization - decinormal solution alkali. Prepared solutions immerse the contaminated spores batistovye test objects. The study of dependence

sporicidal activity of DS, CC and their substances from the pH value

are carried out according to the procedure described above, only when neutralizing The actions of active substances simultaneously reduce the value of pH, adding acid or alkali, respectively.

Determination of the effect of protein contamination on the sporicidal activity DS is conducted in order to identify the possibility of influence (or of establishing him absence) protein pollution on disinfected object for the sporicidal activity of the DS.

The study is carried out using the method of cambric test objects (paragraph 5.8.3.2.), only for the contamination of test objects are used slurry dispute test-microorganism, containing 20% inactivated serum of bovine animals or defibrinated blood, which is added to the suspension at its cooking. Inactivation of normal bovine serum The cattle are carried out by a fractional three-fold heating in a water bath at a temperature of 60 ± 1 ° C for 30 minutes. If the activity of the drug does not decrease in the presence of 20% protein, the concentration inactivated serum of bovine animals or defibrinated blood in the suspension of the test microorganism, increase to 40%. No decrease in sporicidal activity DS at addition and 40% of serum allows to consider DS not reacting to the presence of protein contamination.

1.7.4 Methods for studying the sporicidal efficacy of DS, intended for disinfection of objects of the external environment, contaminated test microorganisms in a spore form

List of test objects modeling objects subject to disinfection, includes: surfaces of premises, furniture, apparatus, devices, sanitary equipment, transport

means, etc .; medical products, including
endoscopes; subjects of nursing, toys; tableware,
laboratory and from under the discharge; Linen, clothes, overalls and other
objects from the tissues; rubber products, including gloves, boots,
aprons, etc .; footwear; Hands in rubber gloves; remnants of food;
allocation: feces, urine, blood, sputum; water; air; medical
waste.

1.7.4.1 Study of sporicidal effectiveness of DS, intended for disinfection of surfaces of premises, furniture, apparatus, instruments, sanitary equipment, vehicles and other objects

In the studies, test surfaces (10 × 10 cm) from
various materials: wood (painted with oil or glue and
other paints; covered with wallpaper and unpainted), linoleum,
plastic, tiles, faience, tiles, metals, glass.

Test-surfaces of various materials (except for
surfaces painted with glue paint and pasted wallpaper)
thoroughly washed with water and soap and a brush, sterilized in a steam
sterilizer. Test surfaces, painted with glue and
wallpapered, wallpapered several times with a sterile gauze
a napkin moistened with sterile drinking water. Prepare a spore
suspension of the test microorganism (B. cereus, anthrax living dry
STI-1 vaccine for humans, B. anthracis) containing $2.0 \cdot 10^9$ spores / ml,
40% horse serum inactivated plus
56 ° C for 30 minutes. (to 6 ml of a suspension containing 2.0×10^9 spores / ml,
add 4 ml of serum).

After drying, the test surface is positioned horizontally and
on them using a single-channel mechanical dispenser or

A glass pipette is applied to a 0.5 ml spore suspension test-
microorganism. The suspension is uniformly distributed throughout the test-
surface (100 cm^2) with a sterile glass spatula. If
the suspension of the test microorganism is not evenly distributed, and
is collected in a drop, grinding with a spatula on the test surface

have (3-5 times). Contaminated
The test surfaces are dried at room temperature until complete
drying (30-120 minutes). Disinfection of test surfaces
are carried out by methods of wiping or irrigation with disinfectant
solution (when working with B. anthracis, only irrigation in
the biological protection box is not pre-dried
more than 20 minutes).

In the behavior of experiments, the test surfaces, colored
glue and other paints, or pasted wallpaper, have
vertically and disinfected by irrigation with disinfectant
solution. The remaining test surfaces are disinfected as in
horizontal, and in vertical positions by irrigation,
single-entry or twofold wiping, or washing
disinfecting solution.

Depending on the type of surface to be treated and availability
contamination on it, the rate of consumption of DS by wiping is
100-150 ml / m ^{2} (1-1.5 ml per 100 cm ^{2}); method of irrigation - 150 ml / m^2
(1.5 ml per 100 cm^2) when treated with a Kvazar spray or its
analogues and 300-500 ml / m^2 (3-5 ml per 100 cm ^{2}) when processing
a sprayer of the "AutoMax" type or a hydro-switch.

If necessary, rubbing or
Irrigation is repeated after 15 minutes.

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For control effectiveness of disinfection across
certain time intervals (15-30-60 minutes) from the test surfaces
Do rinses by thoroughly rubbing the test surface
first in one, and then perpendicular to the direction of sterile
gauze cloth (5 × 5 cm), moistened with a neutralizer. After
Wipe on the test surface should not remain excessive moisture.
Napkins are immersed for 5 minutes. in test tubes (containers) with
corresponding to the neutralizer tested (10 ml),
and then into sterile drinking water with beads and shaken to
shaker for 10 min. The resulting flushing liquid is
0.1 ml on the surface of the nutrient agar of two Petri dishes, carefully

spreading over the entire surface. The crops are incubated in a thermostat at temperature 37 ± 1 ° C for 48-72 hours.

AT control experiments for processing similarly contaminated test surfaces instead of the solution of DS are used Sterile drinking water from the same calculation as the experimental ones. A liquid into which a sterile gauze pad is placed after take the flush from the control surfaces, before planting, dilute in 100 times and 0.1 ml are added to the surface of the nutrient agar of two Petri dishes. The cultures are incubated in a thermostat at a temperature of 37 ± 1 ° C. Take into account the results after 48 hours (preliminary), and the final - after 21 days.

Evaluation of the results of control experiments is carried out on the sowing of A dilution in which the number of CFUs on the surface of the nutrient agar in The Petri dish is from 30 to 300.

After standing in the thermostat, the number CFU on agar plates, the density of contamination is calculated per 100 cm 2 and calculate the effectiveness of decontamination, taking

the number of test microorganisms taken from the control test-objects, for 100%.

For example: on crops with 100 cm 2 of the control test surface found 148,000 CFU, and with a similar type of experimental test-surface - 20 cfu.

148,000 - 100%

20s

x = 20 x 100: 148,000 = 2: 148 = 0.013%

The effectiveness of disinfection of the test test surface is:

100 - 0.013 = 99.987%.

Criterion for the effectiveness of DS in disinfecting test-surface of objects contaminated by the test microorganism in spore form is equal to 100%. If necessary, used in Disinfection modes are subject to approbation on-site facilities.

1.7.4.2 Study of sporicidal effectiveness of DS, intended for disinfection of objects of care of patients and toys of various materials (except soft)

In the studies, test objects (100 cm2) and objects care for patients from various materials: rubbers based on natural and silicone rubber (medical oilcloth, hot-water bottle, pear); glass (drinker, spittoon, thermometer); plastics (heating pad, tray, tip for enemas); metals (a basin, a glass for thermometer); toys (except soft) from rubber and plastics.

Test objects, nursing and toys from various materials are thoroughly washed with water and soap and a brush. Test-Objects are sterilized by steam or air.

A suspension of test microorganism containing 2.0×10^9 spores / ml, to which 40% horse serum is added, inactivated at 56 ° C for 30 minutes. (to 6 ml of suspension add 4 ml of serum).

Using a single-channel mechanical dispenser or glass pipette on the surface of the test object, the object of care for patients, toys are applied 0.5 ml suspension spore test-microorganism. Evenly it is distributed over the surface (100 cm^2) with a sterile glass spatula. Channels and cavities the subject of nursing, toys are filled with a spore suspension using a syringe; small toys are completely immersed in suspension. Contaminated test objects, care items patients and toys are dried at room temperature up to complete drying (60-120 min.).

DS solutions are prepared in drinking water at room temperature temperature. Disinfection is carried out by the immersion method, wiping, irrigation. After drying, the contaminated test objects, nursing items, toys, including

having channels and cavities, are immersed in the solution of the test DS or wipe with a cloth moistened with it. Small toys completely immersed in a container with a solution of DS, preventing them from ascending; large toys are disinfected by irrigation. Consumption rate DS by wiping method at the rate of 100-150 ml / m^2 with a single treatment and 200-300 ml / m^2 with a double; irrigation method - 150 ml / m^2 when treated with a Kvazar spray and 300 ml / m^2 at

spraying of the "AutoMax" type or a water jet. When need for treatment by wiping or irrigation repeat after 5-15 minutes.

For control effectiveness of disinfection across certain gaps time after disinfection (30-60-120 minutes) test objects, nursing and toys remove from solution, do rinses with a sterile gauze cloth (5 × 5 cm), moistened with a neutralizer. Wipes are immersed in sterilized neutralizer solution for 5 minutes, then transferred to test tubes (bottles) with beads and sterile drinking water (10 ml) and shake on a shaker for 10 min. Channels and cavities washed with a neutralizer (10 ml), which is collected in sterile test tubes (containers) and leave for 10 minutes. for neutralization.

The resulting flushing liquid and flush fluid from the channels add 0.1 ml to the surface of the nutrient agar of two Petri dishes and evenly distributed with a sterile spatula on the surface of the medium. In the control experiments, instead of the DS solution, a sterile drinking water.

The cultures are incubated in a thermostat at a temperature of 37 ± 1 ° C. Preliminary results are taken into account after 48 hours, and the final - after 21 days.

Criterion for the effectiveness of DS in disinfecting test-objects, items of care for patients and toys from various materials (other than soft) contaminated with test microorganisms in the spore form 100% death of test microorganisms.

1.7.4.3 Study of sporicidal efficacy of DS,
intended for disinfecting tableware,
laboratory and from under the secretions

For definitions sporicidal effectiveness of DS, intended for the disinfection of dishes, as a test-objects use a set of dining room and tea utensils: plates, glasses, mugs of various materials (porcelain, faience, aluminum, glass, plastic, dishes covered with enamel); cutlery: knives, forks, spoons from a variety of materials (stainless steel, aluminum, plastic), disposable dishes and a set of laboratory utensils, representing test objects of glass and plastics: Subject and cover glasses, pipettes, Petri dishes, plates for immunological analysis and other; crockery (urinals, bedpan). Before experiment, utensils and cutlery is washed with water and soap and a brush, then dried.

As test microorganisms for contamination of dishes The type of dispute most resistant to this DS is used.

On the dishes (100 cm^2 area), pipette 0.5 ml of spore suspension of test microorganisms containing $2 \cdot 10^9$ spores / ml. Culture evenly distributed over the surface of the glassware spatula. Cutlery is immersed in a spore suspension for 1-2 minutes, leaving their pens untreated. Contaminated dishes are dried (until completely dry) when room temperature (60-120 min.) and relative humidity air 50-60% (when working with B. anthracis the dishes are dried no more than 20 minutes.).

For the development of disinfection regimes for dishes with residues food during contamination use a suspension of test microorganisms,

mixed with oatmeal, semolina or other porridge, boiled in milk
with butter (10 g of porridge is added 1 ml of 2-billionth
suspension of a dispute). To simulate the contamination of tea utensils,
jelly (10 ml of kissel add 1 ml of 2-billion suspension of spores),
laboratory dishes - 40% of inactivated whey; utensils
from under the secretions - 20% emulsion of stool, pre-mashed
in a mortar.

Processing of a dining room, tea, laboratory utensils, dining rooms
devices are carried out by immersion in a disinfectant solution.
The solutions are prepared in drinking water. The temperature of the test solution
plus 18-20 ° C. If necessary, study the effectiveness of solutions
DS at a temperature of 50 ± 1 ° C.

The disinfectant solution must be completely filled and
with an excess of covering all dishes and appliances (at a rate of at least 2 liters
for 1 set). Disinfection time of dishes from 15 to 240 min.
depending on the type of test microorganism and the presence of contamination.

After certain time intervals (for example, 15, 30, 60 min.
, etc.) extract one item of different names (for example,
a plate, a glass, a slide, a knife, etc.) from a disinfectant
solution and a sterile gauze cloth (5 × 5 cm) moistened in
solution of the neutralizer corresponding to this DS, carefully
rub the contaminated part of each contaminated
and immersed in 10 ml of the same neutralizer for 5 minutes, then
the napkin is transferred to a test tube with sterile drinking water and beads.
Wash time for gauze napkins is 10 minutes. at constant
shaking. After washing, the gauze napkin is immersed
in the nutrient broth. A wash liquid of 0.1 ml is added to
the surface of the nutrient agar in 2-3 Petri dishes (0.1 ml each

and distribute with a sterile spatula on the entire surface of the medium).
The crops are placed in a thermostat at a temperature of 37 ± 1 ° C. Accounting
the results are carried out after 48 hours for 21 days.

Control is similarly contaminated dishes,
which is not immersed in a disinfectant solution, but in the same

volume of sterile drinking water.

The criterion of the effectiveness of disinfection of contaminated dishes is the death of the test-microorganism spore on the dishes not less than 100%.

1.7.4.4 Study of sporicidal effectiveness of DS, intended for the disinfection of medical devices (MI), including endoscopes

1.7.4.4.1 Study of sporicidal efficacy of DS, intended for the disinfection of medical devices from various materials (except endoscopes)

As test products use sterile tools and medical products, including single use, of different materials (metals, rubbers, glass, plastics) or simulating their test objects. The list of tools should include a variety of forms, the nature of the surface and used material of the product (smooth simple products configuration; Products having locking parts, channels and cavities, having incisions and sputtering, products of crimped form; products, made of several types of materials, etc.).

On the working surface of the test product (for lock products - also in the area of the castle, and in the presence of channels and cavities - also in the channel of the article), 0.1 ml of a suspension containing 2×10^9 spores / ml test microorganism containing 40% inactivated horse

serum. Small test products for contamination are immersed in this suspension for 15 minutes. Contaminated test products are dried in thermostat for 20-25 minutes. When testing funds with fixing properties (eg, aldehydes), serum is added in an amount of 5%.

Disinfectant solutions are prepared on drinking water at room temperature temperature or heated to 50 ± 1 ° C.

After drying, the contaminated products are immersed in solution of the test substance, filling it with all channels and cavities products, avoiding the formation of air congestion. Instruments,

having lock parts, immerse open, previously
making them in the DS solution a few working motions for a better
penetration of the solution into hard-to-reach areas of the product in the area
castle. The thickness of the solution layer above the product should not be
less than 1 cm. In parallel, contaminated products are immersed in
water.

After a certain time (from 15 to 120 minutes), the products are removed
from the solution and a gauze pad measuring 5 × 5 cm, impregnated
neutralizer, the surface of the product make rinses, napkins
is placed in a test tube with 10 ml of the same neutralizer for 5 minutes, then
Transfer it to a test tube with sterile drinking water and shake it off.
beads for 5-10 minutes. To monitor the effectiveness of
Disinfection is done by flushing a 0.1 ml
the surface of the agar
nutrient medium, and the napkin is placed in broth. Product channel
washed with a neutralizer, and the wash liquid is seeded with 0.1 ml
on a dense nutrient medium in Petri dishes. Crops are maintained in
thermostat at a temperature of 37 ± 1 ° C for 21 days. Accounting

preliminary results are carried out after 48 h and final -
in 21 days.

The multiplicity of the statement of the experiment must be sufficient for
obtaining statistically reliable results.

The regime (concentration-time-temperature) is considered to be effective;
providing 100% death of spores of test microorganisms at all
products. In the presence of positive samples, the experiment is repeated,
increasing the concentration or time of exposure.

1.7.4.4.2 Study of sporicidal efficacy of DS,
intended for disinfection of endoscopes

As test objects, fragments of the endoscope or
endoscope (flexible - gastroscope, hard - cystoscope). To 0.1 ml
suspension containing $1 \cdot 10^9$ The test-microorganisms dispute
using a pipette on the outer surface and into the endoscope channel,

dried for 20 minutes. Then the contaminated product
immersed in the solution DS, filling them with cavities and channels of the endoscope.
After a certain time (15 to 60 minutes), the product is removed from the
solution and make flushing from the outer surface of the gauze cloth
(5 × 5 cm), soaked in a neutralizer solution; the napkin is placed in
test tube with 10 ml of the same sterile neutralizer solution
for 5 minutes, and then transferred to a test tube with a sterile drinking
water, and shake with beads for 5-10 minutes. Product channel
washed with a solution of the neutralizer and flushing liquid is sown on
nutritious agar. Conducted also control microbial
seeding of solution used for washing
neutralizer and drinking water. Multiplicity of the experiment

should be sufficient to obtain statistically reliable
results.

The regime (concentration-time-temperature) is considered to be effective;
ensuring the death of the test microorganism on all test products and
absence of test microorganisms in the neutralizer solution. When
The presence of positive samples of the experiment is repeated, increasing
exposure time, but no more than up to 60 min.

Positive is a sample showing a characteristic growth
test microorganism on nutrient media; change in liquid
nutrient medium (turbidity, sediment, flakes, etc.) and characteristic
growth during sowing and re-seeding on a dense nutrient medium, or
detection of the test microorganism in the solution used
neutralizer.

The disinfection mode, developed on channel simulators
endoscope, check at disinfection endoscope,
contaminated by a test microorganism. If necessary
effectiveness of the developed regime is tested in practical
conditions.

Criterion for the effectiveness of DS (mode of application of DS) for
disinfection of medical devices (including endoscopes) is

100% death of the test-microorganism spore.

1.7.4.5 Study of sporicidal effectiveness of DS, intended for the disinfection of linen, clothing, overalls and other objects from the fabric

Studies with DS are conducted to assess the effectiveness of its for decontaminating laundry and other objects from the fabric, clean and contaminated with blood or secretions (feces, urine, sputum).

Evaluation of the effectiveness of DS for disinfection of clothes, clothes, Work clothes and other objects from the fabric are carried out with the help of Sterile test objects, which are pieces of calico size 2 × 2 cm. Calico is pre-cooked and disinfected as well, as a cambric. Sterile test objects are contaminated with a suspension of test-microorganisms containing $2 \cdot 10^9$ spore / ml, at the rate of 20 ml for 10 test objects. After 30 minutes. test objects are laid in coarse calico pouches measuring 5 × 8 cm (2 strains each) that cover in the form of an envelope.

The solution of the tested DS on drinking water is room temperature temperature or heated to 50 ± 1 ° C is prepared at the rate of 5 liters per 1 kg linen. Rags, imitating linen, are individually poured into a container with solution of the tested DS so that between the layers of tissue is not formed air strata that impede the process disinfection. Simultaneously, between the layers of laundry are distributed (top, middle and bottom) bags with contaminated test objects. After a given time, the bags are removed simultaneously from three layers. Test objects are taken out of the bag with sterile tweezers, place for 5 minutes. in a container with a solution corresponding neutralizer, then transferred to a sterile drinking water and plated in a hotting broth (pH 7.2). Crops incubate at 37 ± 1 ° C for 48 hours. Preliminary accounting the results are carried out after 48 hours, and the final one - after 21 days. In control experiments, the laundry is immersed in sterile drinking water. Pouches with tests are laid in the same way as in the experiment. When receiving 100% death of the test microorganism in experiments on disinfection of the laundry

Without protein contamination they pass to experiments on disinfection
laundry, contaminated with secretions.

To determine the effectiveness of DS in the disinfection of laundry,
clothes, overalls and other objects from tissue contaminated with blood,
secretions (feces, sputum, urine, etc.), in the laboratory
use coarse test objects that are contaminated by calculation
30 ml of suspension for 10 test objects with test spore suspension test-
microorganism with the addition of 40% inactivated serum
(6 ml of a suspension containing 2 × 10 9 test-microorganism spore
mixed with 4 ml of inactivated whey) or 40% of fecal
emulsion (6 ml of spore suspension of the test microorganism are mixed with 4 ml
40% fecal emulsion). For the preparation of a fecal emulsion, 8 g
The faeces are ground in a mortar with 20 ml of water. The amount of spore suspension
A test microorganism containing serum or feces is prepared from
calculating 30 ml for 10 test objects. Contaminated test objects
dried in a thermostat at 37 ± 1 ° C for 20-25 minutes. or
1.5-2 hours at room temperature until completely dry.
The experimental procedure is similar to the experiments with pure
underwear.

Criterion for the effectiveness of DS in the disinfection of laundry,
clothes, overalls and other objects from the tissues is 100% doom
the test-microbial spores at test objects.

When studying the effectiveness of disinfection of products from
synthetic fabrics (kapron, acetate, periacetate, lavsan, etc.)
use test objects from these tissues measuring 5 × 5 cm, since
microorganisms do not penetrate into the structure of these tissues and washability
they are 2 times more than from the batist test objects.

1.7.4.5.1 Study of the sporicidal efficiency of the chamber method disinfection

The chamber method is used to disinfect clothes, shoes, bedding, soft toys, etc.

As a test microorganism, B. cereus is used in the form of a suspension containing 2×10^9 spores / ml, which is contaminated with test-objects from cambric, coarse calico and other materials corresponding to disinfected objects. Contaminated test objects put in sterile envelopes of cotton fabric (2 test object in the envelope). Numbered test objects are placed in the Cotton bags with maximum thermometers and placed in the thickness of objects in the camera control points on three levels.

After disinfection, the bags are removed from the chamber and recorded the maximum thermometers, and the test objects are placed in the test tubes with 5 ml of nutrient broth.

Incubation of crops with test objects is carried out at temperature 37 ± 1 ° C for 21 days using nutritional broth (bouillon Hottinger, SPB, BCH). The preliminary recording of the results is carried out after 24-72 hours, the final one - after 21 days. To establish the effectiveness of processing is carried out at least three experiments for each time processing. In the presence of growth of microorganisms, a comparison is made grown culture with a test microorganism.

AT quality control use contaminated test objects that were not placed in the camera. Control cultures are grown using the same culture media as for experienced test samples. Control cultures and media control

similar to the test objects that were processed in the chamber. For To establish the effectiveness of treatment, at least three experiments for each treatment time.

Controlling the effectiveness of the disinfection of

The test disinfection chambers are 100% death spores
test microorganisms.

1.7.4.6 Study of sporicidal efficacy of DS
when disinfection of hands in rubber gloves

As a test microorganism, a lyophilic-
dried anthrax dry vaccine STI-1. Prepare a spore
suspension by applying in a vial with a dry vaccine 2 ml sterile
distilled water. After dissolving the dry vaccine,
a suspension containing (1.0-5.0) · 10^8 spores / ml, diluted to
content 10^3 , 10^5 and 10^7 spores / ml.

To eliminate extraneous microflora of the hand, including wrists
and forearms, the testers carefully wash with soap in a warm flowing
water, then wipe with a sterile gauze napkin and dress
latex gloves.

The surface of rubber gloves worn on the hands of testers-
volunteers, are contaminated by thorough trituration of 1 ml
spore suspension with the above three dilutions (each
breeding for one tester). After the microbial suspension has dried
to control the initial seeding from the rubber surface
The gloves of the back of the hand make the washing away a sterile gauze
a tissue 5 × 5 cm, moistened with sterile drinking water.
Then the napkin is placed in a test tube with 10 ml of sterile drinking

water with beads and shaken for 10 minutes. The resulting wash is sown by
0.5 ml into a selective liquid medium (9.5 ml) in a test tube.

To disinfect the surface of gloves in the compressed palm of the hand
In the test-volunteer, 2.5 ml of the test
sporocidal DS.

Then it is within 10-15 seconds. wipes this portion
disinfectant solution surface of gloves of both hands,
making movements of the hands that are performed while processing the hands skin
antiseptic. After this, the same operation is carried out by applying 2.5 ml

disinfectant solution on the palm of the second hand and stopwatch the beginning of the exposure.

After a 5-minute exposure, rinse with a gauze cloth (5 × 5 cm), moistened with a suitable DC neutralizer, previously tested for neutralization efficiency and static action on spores. The napkin is placed in a test tube with 10 ml sterile neutralizer for 10 min. Then a napkin is transferred with sterile tweezers into a test tube with 10 ml of sterile drinking water with beads, shake the tube 10 min. in the shaker. From the resulting flushing, a 0.5 ml crop is made for selective liquid nutrient media in test tubes (at least 3 test tubes per sample).

The mode of application of DS providing 100% death of the investigated STI-1 vaccine spore on rubber gloves, protecting the skin of hands.

1.7.4.7 Study of sporicidal effectiveness of DS, intended for water disinfection

Methods extend on study sporicidal effectiveness of chemical DS in disinfection of drinking and

Natural water containing or suspicious for maintenance the causative agent of anthrax.

When determining the sporicidal effectiveness of DS as a test-objects use plumbing (dechlorinated), well, river and other water. Tap water is dechlorinated by heating to temperature of 50-60 ° C, followed by keeping for some days at room temperature. Determine the physico-chemical properties of drinking dechlorinated water and samples of natural waters. In addition, the latter determine the microbiological indices (total microbial number and total number of coliform bacteria).

As test microorganisms for contamination of the investigated water samples using a spore culture of B. cereus or anthrax live dry STI-1 vaccine for humans. Virulent To this end, the culture of the causative agent of anthrax

is permitted due to the complexity of antiepidemic
activities.

The contamination of water samples under study is carried out by introducing a spore culture of B. cereus in the form of a suspension containing 10^8 spores / ml, and the anthrax living dry vaccine STI-1 - in the form of a suspension prepared by diluting the contents of one ampoule vaccine in 10 ml of sterile saline or sterile tap water to a content of 10^8 -10^9 spores / ml.

When the experiments are set, the initial water is poured into containers with a lower tube of 5-10 liters and added to water the above test microorganisms at the rate of 10^5 -10^6 spores / l. After The concentration of the initial contamination of water. For this, 1-3 water samples are taken from the containers volume of 3-5 ml. From each sample, 3-4 consecutive

tenfold dilutions with sterile saline or sterile drinking water, in which the amount of test microorganism in 1 ml of water sample by membrane filtering.

The method is based on the concentration of microorganisms from a certain volume of water to be analyzed by filtration through membrane filters, cultivation of crops at a temperature of 37 ± 1 ° C on a dense nutrient medium, and counting the number of test-microorganisms per unit volume of water.

Membrane filters are used for microbiological purposes with a pore diameter of not more than 0.45 μm and a disc size of 35 or 47 mm or Other filtering membranes with similar capacity filtering, having a quality certificate. Membrane filters prepare for analysis in accordance with the manufacturer's instructions.

The filtration of water is carried out using a device for membrane filtering. A glass (funnel) and a table of the device before analyzing the water wrapped in paper and sterilized in steam or air sterilizer. On the lower part of the device (table) flamed tweezers sterile membrane filter; press it with the upper part of the device (glass, funnel);

fixed with a device provided by the design of the device; at
The observance of the rules of sterility poured the necessary volume
of the investigated water and create a vacuum in the receiving vessel.

First, smaller and then large amounts of water
through one filtering device, each time replacing the membrane
filter.

After the filtration of a certain amount of water
The glass (funnel) is removed, and the filter is gently lifted off the edge

flambied tweezers while maintaining vacuum for removal
excess moisture on the underside of the filter, and then transfer it, not
turning over, on the surface of casein or meat-peptone agar
in Petri dishes, so that there is no filter between the medium and the filter
air bubbles. Under each filter on the back of the bottom
Petri dishes make an inscription indicating the volume of filtered
water, date of sowing and sample number. Crops are incubated at
temperature 37 ± 1 ° C for 24-48 hours.

At the end of the incubation, the number of test-
microorganisms grown on filters, and determine their
concentration in 1 liter of water according to the formula:

$$C = \frac{k}{v} \times N \times 100,$$

Where

C - the number of spores contained in 1 liter of water;

k is the multiplicity of the dilution;

v - sown volume of water in ml;

N is the arithmetic mean of the number of colonies grown on
membrane filters when sowing the same dilutions.

The result of the analysis in determining the number of spores in the source water
is expressed by the number of CFU per liter of water.

To determine the effectiveness of sporicidal disinfection
DS in a container of water, contaminated with test-
microorganisms, make DS in the concentrations studied, water
mix thoroughly. At predetermined intervals of time,

Observing the conditions of sterility, take samples of water with a volume of 1 liter in sterile bottles with a sterile neutralizer introduced into them,

selected in a concentration that provides neutralization acting agent of the DC being studied.

In disinfected water determine the number of non-fatal spores B. cereus or anthrax alive STI-1 vaccine for humans using the membrane filtering. Samples disinfected water from neutralizer should be investigated no later than 1 hour later after their selection.

At the initial stages of studying the effectiveness of disinfection water analysis of at least two samples differing in volume in 10 times, and selected so that on one filter there is no more 300 colonies. For one Petri dish, you can put 3-4 filters with the condition that they do not come into contact with each other. In the analysis disinfected water at the final stages of decontamination To investigate volume not less than 1 liter, filtering this quantity not less than 3-4 membrane filters.

The crops are incubated as above. Take into account the general the number of colonies grown on filters after filtering 1 liter water. The result of the analysis is expressed by the number of spores in 1 liter of disinfected water.

Statistical processing of microbiological analysis of water disinfection effectiveness aims to exclude random errors, estimate deviations results of the analysis from the actual value and give the desired results with a given probability.

AT process statistical processing the results experimental research is recommended to use Determination of the concentration of contamination of the source water test-microorganisms and at intermediate stages of processing the average

arithmetic (X), and in assessing the effectiveness of decontamination on the final step is the median value (Me).

The number of trials n required for a valid estimate The results of microbiological studies are determined by formula:

$$n = \left(\frac{\sigma t_p}{I_p} \right)^2$$

Where

σ is the quadratic deviation;

I_p - the maximum allowable deviation from the average, estimated with probability p = 0.99;

t_p is a coefficient that depends on the number of experiments (at least 10), on which determines the value of σ.

If σ is determined from the data of 16 experiments, then $t_{0.99} = 2.7$. Number of samples to assess the content of microorganisms in disinfected water must be at least 16.

Evaluation of the results of microbiological analyzes is carried out for given the probability of 0.99, respectively, for the same value determine the confidence interval of the arithmetic mean and medians.

The confidence interval of the arithmetic mean is determined by given the value of the quadratic deviation σ and the mean error σ_x.

The magnitude of the quadratic deviation is calculated by the formula;

$$\sigma = \frac{\Sigma (x - X)^2}{n-1}$$

where $\Sigma (x-X)^2$ is the sum of the squared deviations of the measurements from the mean arithmetic, and n is the number of individual dimensions.

The average error is calculated by the formula:

$$\sigma_x = \frac{\sigma}{\sqrt{n}}$$

The probability of 0.99 corresponds to the confidence interval I, calculated by the formula: $I_{0,99} \pm 2,7\sigma_x$

Then the confidence value of the number of microorganisms in sample with a probability of 0.99 lies in the interval $X \pm 2.7\sigma_x$.

The median confidence interval for the required level Probabilities 0.99 are determined depending on the number of Experiments on the table, in which the numbers of the experiments are indicated, the results which are taken into account as boundary values of the trust median interval.

To use the table, it is necessary that the results of the experiments were arranged and numbered in ascending order of magnitudes.

Criterion for assessing the effectiveness of DS in water disinfection is the lack of test microorganisms in 1 liter of water.

Table 1.11.

Media Confidence Intermediate Limits

Number experiments	Lower border	Upper border	**Number experiments**	Lower border	Upper border
7th	-	-	**29**	8	22
8	1	8	**thirty**	8	23
9	1	9	**31**	8	24
10	1	10	**32**	9	24
eleven	1	eleven	**33**	9	25
12	2	eleven	**34**	10	25
13	2	12	**35**	10	26th
14	2	13	**36**	10	27th
15	3	13	**37**	eleven	27th
16	3	14	**38**	eleven	28
17th	3	15	**39**	12	28
18	4	15	**40**	12	29
19	4	16	**41**	12	thirty

20	4	17th	**42**	13	thirty
21	5	17th	**43**	13	31
22	5	18	**44**	14	31
23	5	19	**45**	14	32
24	6th	19	**46**	14	33
25	6th	20	**47**	15	33
26th	7th	20	**48**	15	34
27th	7th	21	**49**	16	34
28	7th	22	**50**	16	35

1.7.4.8 Study of sporicidal efficacy of DS, intended for decontamination of secretions

(urine, feces, sputum), biological fluids (blood)

Disinfection of urine.

As a test object, urine is used, which is poured into flasks or tubes of 8 ml, add 1 ml of spore suspension, containing $2 \cdot 10^9$ spores / ml of the test microorganism.

The solutions of the tested DS are prepared in concentrations, sporicidal activity when tested for batistovyh test objects with protein protection.

The test concentrations of DS solutions are added to the urine in equal or double volume. Note the time of contact and through intervals of 15, 30, 60 minutes. pipette, the mixture is taken in an amount of 1 ml and transferred to test tubes with 5 ml of nutrient medium (broth Hottinger, pH 7.2) and an appropriate neutralizer. After thoroughly mixing 1 ml of liquid from the first test tube with broth is transferred to a second tube with broth (5 ml) and then are inoculated with 0.1 ml per Hottinger agar (pH 7.2) in Petri dishes, as from first, and from the second test tube. Petri dishes are incubated in a thermostat at 37 ± 1 ° C.

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Control is similarly performed experiments with only adding to the urine is not a disinfectant solution, but water.

An approximate recording of the results is carried out after 1 day, preliminary - after 5-7 days, final - 21 days.

The results of the experiments are taken into account in relation to the control which take for 100%. The final conclusion on the effectiveness of DS make on the basis of not less than three experiments with coincident results.

An effective means and mode of its application is considered effective, providing 100% death of spores of test microorganisms.

Disinfection of feces.

In the development of disinfection regimes, feces are taken into account

the ratio of the disinfectant to the disinfected mass,
processing time, temperature, consistency of disinfected

the degree of homogenization in the process of disinfection.

Research is carried out in two stages. At the first stage, as

test-object use 20% emulsion of feces, on the second -

decorated feces.

To prepare a 20% emulsion, 20 g of fecal matter is ground

in a mortar and add 80 ml of water; the resulting emulsion is filtered

through a double layer of gauze, sterilized in an autoclave, poured

pipette into a 9 ml vial and add 1 ml of the suspension

test microorganism containing $2 \cdot 10^9$ spores / ml. Experiments begin with

concentration, causing the death of a test microorganism in the urine with

protein after 30 minutes. The prepared emulsion of feces is poured

equal to or double the volume of disinfectant solution,

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after 30, 60, 120 minutes. Samples are taken and sowings are made in the same way as

and with disinfection of urine. The results are taken into account after 48 hours.

With positive results, experiments with large

number of decorated feces (200-250 g). To do this, place them

in a vessel, pour a disinfectant solution or fall asleep dry

disinfectants in equal or double amounts in relation to

weight of feces, determine whether the homogenization occurs visually

faeces. Then a small part of stool is stirred

a glass rod with a liquid, and the remaining mass is left in the form

small lumps. At regular intervals

(for example, 30, 60 minutes) are sowing.

Sowing of the liquid part of the feces is sown in the same way as urine.

Dense parts (lumps) are taken with a bacteriological loop and

is placed in 5 ml of culture medium with the appropriate

neutralizer, rastering them on the edge of the tube and thoroughly mixing.

Then 1 ml of the mixture is transferred from this tube in a sterile pipette

The second tube, also containing the Hottinger broth (pH 7.2). how

from the first, and from the second test tube, the agar is sown

Hottinger in Petri dishes (not less than three cups of 0.1 ml each).

Final result take into account across seven days, and preliminary - in 24 hours.

Control is similarly performed experiments with adding water instead of a disinfectant solution. results Experiments are taken into account in relation to the control that is taken for 100%. Judge the effectiveness of the test substance on the basis of less than three experiments with coincident results. Effective consider the means and mode of its use, providing 100% death of spores of test microorganisms in the disinfected material.

Disinfection of blood and sputum.

As test objects in the evaluation of sporicidal efficacy DS destined for blood disinfection uses blood, and phlegm - chicken protein. As a test microorganism, disputes B. cereus. For contamination test objects test-microorganism to 9 ml 40% blood or 50% chicken protein added 1 ml of a suspension of test microorganism containing $2 \cdot 10^9$ spores / ml, mix and pour 1 ml into sterile bottles. Then in bottles fall asleep or pour the investigated DS in bulk (5%, 10% etc.) the ratio to the volume of the material being studied. Across certain time intervals (1 hour, 3 hours, etc.) with the help of sterile bacteriological loop select the sample of the mixture and is transferred to 5 ml of a selective liquid culture medium with relevant (in advance proven by effectiveness of neutralizing the DS) with a neutralizer to neutralize the residual action of DS on the spores of the test microorganism. After 5 minutes. Exposures from this tube are pipetted by sowing 0.2 ml of the test sample for the Hottinger agar (pH 7.2) in Petri dishes. Tubes and plates with inoculations are incubated at 37 ± 1 ° C.

The final result of growth of test microorganisms on plates take into account for 3-7 days, and preliminary - in 24 hours, and in test tubes, respectively, after 21 days and 24-48 hours.

An effective means of ensuring 100% death is considered effective The test-microorganism spores in the disinfected material.

1.7.4.9 Study of sporicidal efficacy of DS, intended for the disinfection of medical waste

When studying the sporicidal activity of DS with the aim of developing Disinfection of medical waste uses test-objects from rubbers, plastics, textile materials, glass, metals. For the preparation of test objects, sterile disposable medical products (bandages, cotton swabs, fragments of systems for blood transfusion and medicinal products, catheters, spatulas, syringes, needle gloves, disposable underwear, napkins, pipettes, tubes and the like) are ground and immersed in a slurry containing 2×10^9 spores / ml B. cereus with 40% inactivated horse serum or serum of cattle. After enough impregnation, the test objects are removed to a dry sterile container and dried in a thermostat for 20 minutes. or at room temperature temperature at 18-20 ° C and relative humidity 50-60% for 1 hour.

The contaminated test objects are immersed in a container with disinfectant solution, so that it is completely closed them. Control of the effectiveness of disinfection of test objects spend every 15-30 minutes. for a period of 30 to 360 minutes. To do this, test objects from different materials (two each denomination) is removed from the disinfecting solution, washed in a solution of the appropriate neutralizer and placed in tubes with liquid nutrient medium. Control test objects are immersed in the maximum exposure in sterile drinking water, and then in liquid nutrient medium. Tubes with crops are placed in thermostat at a temperature of 37 ± 1 ° C. The results are recorded in for 21 days.

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Criterion effectiveness of disinfection medical waste - 100% death of the test-microorganism spores at test facilities, processed DS.

1.7.5 Methods of research and evaluation of sporicidal activity of DS when used as test microorganisms virulent strains of B. Anthracis

When a test is used as a test microorganism non-pathogenic strains (B. cereus, B. subtilis, anthrax alive dry vaccine STI-1, etc.) the sporicidal activity of the DS is determined only by the culture method, and when using virulent B. anthracis - at the same time as culture and biological. With the culture method, the results of the studies are evaluated by the growth of test microorganisms on nutrient media before and after the action of DS, and in the biological - on the death of white mice from the causative agent of anthrax.

The difficulty of observing antiepidemic The use of virulent B. anthracis strains does not allow Use it to determine the effectiveness of disinfection Many objects (water, air, chamber method of disinfection things, etc.), therefore, to study sporicidal activity and effectiveness of DS in decontamination of all objects that are factors of anthrax pathogen transmission, it is recommended that use non-pathogenic test microorganisms.

After the establishment of sporicidal activity of DS with a culture method, applying the above non-pathogenic test-microorganisms, expediently spend research culture and biological methods, using B. anthracis, both

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as a biological method makes it possible to distinguish the sporicidal effect of DS from sporostatic, in the absence of complete neutralization

active substance DS with chemical neutralizers.

Sporocidal efficacy of DS with use of spores virulent strains of B. anthracis can be studied in the following contaminated test objects: surfaces of premises, furniture, apparatuses, devices, sanitation equipment, Vehicle; medical products; subjects care for patients; Tableware, including table, laboratory and from excretions; linen, overalls and other objects from fabrics; products from rubber, including gloves, boots, aprons, etc .; excretions (feces, urine), medical waste.

Methods for studying the sporicidal activity of DS at work with B. anthracis. As test microorganisms are used virulent strains of B. anthracis (strains 81/1 and 27 having plasmids pX01 + and pX02 +) whose single cells at intraperitoneal administration cause the death of bioprogenic animals. Characteristics of these strains, stability requirements, nutrient media for cultivation are given in 5.8.1. and applications 1 and 2.

Methods for obtaining spore suspension of virulent strains B. anthracis, ensuring standard conditions for conducting studies of sporicidal activity of DS and their substances, Studies of sporicidal activity and efficacy in the development of regimes of using DS for disinfection of external objects The environment contaminated by test microorganisms in a spore form, are stated in clause 1.8.1.

When controlling the sporicidal effect of DS on spores of virulent strains of B. anthracis, the active ingredient is needed immediately after graduation exposition neutralize relevant neutralizer, followed by sowing on solid and / or liquid nutrient media (as described in the previous sections).

At the same time, part of the sample is examined in the setting of a bioassay, using white mice weighing 10-12 g in the amount of 6 pieces per

setting a single bioassay. Prepared and used sample
is administered intraperitoneally to white mice in a volume of 0.2 ml each.
Simultaneously, the animals are subjected to control solutions of the solution
neutralizer (control of the absence of detrimental effect
neutralizer on white mice) and a suspension of intact
exposed to DS) spores of virulent strains of B. anthracis
in infectious doses (control of the virulent action of the used
suspension of spores of the pathogen). Number of animals in control
groups must be at least 3.

Observations of the animals are carried out for 48-96 hours;
dead and surviving mice are opened, sowing is done from the organs
animals to a Hottinger agar (pH 7.0) to isolate the pathogen
anthrax.

Crops of prepared samples from animal organs are incubated
at a temperature of 37 ± 1 ° C for 48-96 hours. Identify and
count up the grown colonies of B. anthracis.

An effective DS is considered effective, after exposure to which in
established regimens there are no viable cells
virulent test microorganisms B. anthracis in the study
culture method and there are no dead animals in the experimental group, and in

control samples - sowing out of the organs of animals a pathogenic test-
microorganism (B. anthracis).

1.7.6. Methods of research and evaluation of sporicidal efficacy
sterilizing agents intended for sterilization
Medical devices, including endoscopes

1.7.6.1 Study of the sporicidal efficiency of water
saturated vapor under excessive pressure, intended
for sterilization of medical devices

Method is intended for research effectiveness of
sterilization of medical products by saturated steam
excessive pressure, including during testing of new steam
sterilizers. The study is carried out in steam sterilizers at
loaded sterilization chamber.

To control the effectiveness of sterilization of medical devices
water saturated steam under excess pressure is used
test objects and test products, contaminated with spore suspension test-
microorganism G. stearothermophilius, as well as biological
indicators approved for use in the established manner, with
steam sterilization method in the Russian Federation.

As test objects, test samples (0.5 × 1.0 cm) are used,
cut from rubbers, latex, calico, dressings, etc.
Prepared test objects are sterilized by steam or air
method (test objects from rubbers, latex, calico, bandages
materials are laid out in Petri dishes).

From the initial suspension, spores of G. stearothermophilius are prepared
a suspension containing from 5.0×10^{7} to 2.5×10^{8} spores per ml for
contamination of test objects. Sterile test objects contaminate
This suspension is calculated from $(1.0\text{-}5.0) \times 10^{6}$ spores per object by applying

for each test object using a variable volume dispenser
0.02 ml of the prepared suspension. Contaminated test objects
is dried in a thermostat at a temperature of 37 ± 1 ° C for 24 hours and
pawn in paper and combined (polymer film +
paper) packages approved for use as
sterilization packaging materials for the steam method
sterilization in the Russian Federation.

As test products use medical products from
corrosion-resistant metals (surgical, dental,
gynecological instruments - forceps, clamps, forceps,
tweezers), glass (test tubes, micropipettes), rubber (catheters, probes,
tubes), plastics (tips, trays), which are preliminarily
I sterilize the vapor method, and then contaminate the original
spore suspension of the test-microorganism G. stearothermophilius (density
contamination of 10^{6} spores per test product) and packaged in paper
Packages or sheet materials, or calico - when placing products in
sterilization boxes; in combined packages - with
placing products in the baskets of loading.

Packages with contaminated test objects / test products,
as well as biological indicators are numbered and placed together with
maximum thermometers in the sterilization control points
cameras outside the sterilization boxes / boot baskets at the door and
back wall and in sterilization boxes / baskets loading
between products.

Test objects are placed between products used in
as imitators of loading, as well as in products - gloves, gowns;
test products - between products used as
load simulators.

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Density of box loading with sterilization products from
Rubber and textiles must comply with the standards given in
"Methodological instructions for disinfection, pre-sterilization
purification and sterilization of medical devices "
(MU No. 287-113 of 30.12.1998). These products, as well as products from
metals and glass in sterilization boxes and baskets
loading, distribute evenly; packages are filled with products
not more than ¾ of the volume.

The sterilizer is switched on and after it is released to the mode
(attainment nominal meanings pressure / temperature
sterilization), the exposure time starts. At the end
Exposure time test objects and test products are taken out of
sterilizer.

Test objects from rubbers, latex, calico, bandages, tweezers,
which is burned in a flame, placed in a bacteriological
test tubes with 5 ml of nutrient broth (Hottinger broth, MPB, SPB).

To control the sterility of large test products subjected to
processing in a sterilizer, seeding in a nutrient broth
gauze pads (size 5 × 5 cm), with the help of which produce
Sampling from products by flushing. Napkins
moistened with sterile saline (0.9% solution
sodium chloride). In products having channels, through the last
the physiological solution is passed, after which it is sown in

nourishing broth.

Incubation of the crops is carried out at a temperature of 55 ± 1 ° C for 7 days with the use of nutrient broth (broth Hottinger, SPB, BCH).

In the presence of growth of microorganisms, a comparison is made of the isolated culture with the test microorganism.

As controls, test objects and test-products that are not processed in a sterilizer. Crops control test-objects and test-products or wash-off from them in The nutrient medium as well as the incubation of the crops are carried out similarly test objects / test products, which subjected processing in the sterilizer.

A similar methodology for research is used effective modes of sterilization of medical devices in newly developed steam sterilizers. For this experiments are carried out at different holding times and placement of test objects / test products and maximum thermometers in different points of the sterilization chamber. To establish at least three experiments (each mode) every processing time.

The efficiency criterion of water saturated steam under excess pressure for sterilization of medical devices 100% death of G. stearothermophilius spores at test sites and test-products (test objects / test-products) from various materials, processed in the apparatus under study.

1.7.6.2 Study of sporicidal effectiveness of dry hot Air intended for the sterilization of medical devices

Method is intended for research effectiveness of sterilization of medical devices with dry hot air, including number when testing new air sterilizers.

The study is carried out in air sterilizers with
loaded sterilization chamber.

To control the effectiveness of sterilization of medical devices
dry hot air as test products use test tubes
type P2-21-200, GOST-25336-82 (for sterilizers with a volume of
sterilization chamber up to 20 dm ^{3} inclusive) and cups
biological Petri with lids low ЧБН-1-100 in accordance with GOST-25336-82
(for sterilizers with a sterilization chamber volume of 40 dm3 and
more). Pre-sterilized steam or air
method and contaminated with a spore suspension of the test microorganism
B. licheniformis. In addition, biological indicators are used,
allowed for use in accordance with the established procedure, with air
method of sterilization in the Russian Federation.

From the initial suspension of B. licheniformis spores, a suspension is prepared,
containing from 10 ^{8} to 10 ^{9} spores in 1 ml for the contamination of test products
at the rate of 10 ^{6} spores in / on the test product, which is achieved by applying to / on
each test product with a variable volume dispenser of 0.02 ml
prepared suspension.

The contaminated test items are dried in a thermostat at
temperature 37 ± 1 ° C for 24 hours, then them and load simulators
(test tubes and Petri dishes) are packaged in sheet paper,
in paper or polymer bags approved for use in
quality sterilization packaging materials for air
method of sterilization in the Russian Federation (test tubes of 1 strain,
Petri dishes - 2 strains per package).

Packages with test products, as well as biological indicators
Numbered and placed on the loading shelves in the accommodations
temperature sensors (not less than 5 points). Download simulators

evenly placed on the loading shelves of the sterilizer

(the number of load simulators, including test products) in
depending on the volume of the sterilization chamber is:

20 dm 3 - 40 tubes; 40 dm 3 - 40 Petri dishes; 80 dm 3 - 80 cups
Petri; 160 dm 3 - 160 Petri dishes; 320 dm 3 - 320 Petri dishes;
640 dm 3 - 640 Petri dishes.

The sterilizer is also included after the nominal
The values of the sterilization temperature begin to count down
sterilization exposure. At the end of the sterilization time
Exposure test items and biological indicators are taken out of
sterilizer. Tubes are filled with a nutrient broth (broth
Hottinger, BCH, SPB); Petri dishes - nutrient agar (agar
Hottinger, IPA, SPA).

Incubation of test products is carried out at a temperature of 37 ± 1 ° C in
for 21 days with the use of nutrient broth (broth
Hottinger, BCH, SPB); Petri dishes are incubated for 7 days.
The results are recorded by visual inspection.

Absence of opacities nutritional broth, growth
microorganisms on the petri dish indicates the death of the spores in / on
test-products. In the presence of growth of microorganisms
comparison of the isolated culture with the test microorganism.

As controls, test products are used that do not
is subjected to treatment in a sterilizer. Crops of control
test products or wash them off to a nutrient medium, and
Incubation of crops is carried out similarly to test products,
which were processed in a sterilizer.

A similar research methodology is used for
effective modes of sterilization of medical devices in

sterilizers of different brands, including in newly developed
sterilizers. For this purpose, the experiments are carried out for different
time of exposure and placement of test objects and test products,
temperature sensors at various points in the sterilization chamber.
To establish the efficiency of the treatment, at least three
experiments for each treatment time.

The criterion for the efficiency of dry hot air for sterilization of medical products is 100% death spores

B. licheniformis on the test objects processed in the test apparatus

and test products from various materials.

1.7.6.3. Studies of the sporicidal effectiveness of solutions chemical SS, intended for sterilization of medical products

In the study and evaluation of sporicidal efficacy

sterilizing agents for sterilization

medical products use B. cereus and B. subtilis with a certain

stability (Table 5.8.).

Cultivation spore-forming test microorganisms,

resistant to the active substance that is part of the studied

sterilizing agent, preparation of spore suspension (5.8.1.).

As test carriers, sterilized test-

samples from various materials (plastics, rubber based on

natural and silicone rubber, glass, metals), imitating

medical product, as well as medical products themselves (catheters,

burs and dental mirrors, clamps, endoscopes). For

contamination of the test carriers use the spores of the test microorganism,

which has the greatest resistance to a solution of this

chemical agent.

When testing a solution,

studied for this purpose the active substance, select

A stable spore-forming microorganism whose spores can

be used as a test microorganism (paragraph 5.8.3.).

For the purpose of contamination of test products, drip them

A suspension of test-microbe spores is applied (based on 106 spores per

test product). Test microorganisms should be applied to

the most complex in design and hard-to-reach areas of test-

products: in canals, workers and lock parts. After this test product

dried for 60 minutes. at a temperature of 37 ± 1 ° C.

During the experiments, the products are immersed in working
Solutions in such a way that the solution completely covers the products
(the thickness of the solution layer above the surface of the products must be
not less than 1 cm) and filled all cavities and channels without air plugs.
Instruments with lock parts must be made
several working movements for better penetration of the solution
means in the field of the castle. In the studies used solutions,
having a room temperature of 18-20 ° C. When testing solutions
aldehyde-containing agents, the temperature of the solution should be
plus 20-22 ° C. If necessary (to increase activity
solutions) are prepared working solutions of moderately elevated
temperature plus 50 ± 1 ° C. At the same time, at least two test-
the object is placed in water, as well as in the neutralizer solution, for a time,
corresponding to the maximum exposure time
in the solution being studied.

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The study of the effectiveness of the agent and its working solutions in
depending on the shelf life, as well as (if necessary) the study of
efficiency of working solutions with repeated use
are carried out in the same manner as above, using solutions
funds with different storage times and frequency of use
for sterilization.

When working with test objects and products of small size
(burs) at regular intervals (depending
from the composition of the agent), the products are removed from the solution of the test
and sequentially washing in solution
neutralizer and sterilized drinking water (5 minutes each) with
followed by direct seeding on liquid nutrient media with 0.5%
glucose.

When working with large products to control sterility
treated products
gauze pads (size 5 × 5 cm), with the help of which produce
Sampling from products by flushing. Napkins

moistened with a solution of the appropriate neutralizer. When washing products having channels, pass through the latter a solution neutralizer. If the neutralizer for the test substance is not It is known, it is expedient to use a universal neutralizer, tested for efficiency. The crops are kept in a thermostat at temperature 37 ± 1 ° C for 14 days.

The criterion of effectiveness is to achieve 100% death of the dispute test-microorganism on all test-products. In this case, the action time solution should not be more than 16 hours at a temperature 18-20 ° C, and no more than 3 hours at a moderately elevated temperature (50 ° C).

1.7.7. Research and evaluation of sporicidal efficacy sterilizing agents for research efficiency of disinfection of high level endoscopes

Means intended for high level disinfection endoscopes, should ensure the death on endoscopes of spore forms microorganisms.

In the study, test objects are used sterilized fragments of the flexible endoscope channel in the form plastic tubes 20 mm long and 2 mm inside diameter. Before the experiment, the test objects are cleaned one of the means allowed for pre-sterilization cleaning endoscopes, and then sterilized by physical (steam) or chemical method recommended for the sterilization of flexible endoscopes.

The sterilized test objects are artificially contaminated, applying by means of a dispenser / micropipette to the central part of the channel and on the surface of each tube a spore suspension of the test microorganism (p. 1.8.4.4.2.) from the calculation of 106 spores per each test object. The contaminated test objects are dried at a temperature of 37 ± 1 ° C for 120 min.

The treatment with the test agent is carried out by a method immersion in the solution under study, having a temperature of 18-20 ° C (for aldehyde-containing products - 20-22 ° C). In carrying out

experiments, test products are immersed in working solutions such
so that the solution completely covers the product (the thickness of the layer
solution above the surface of the products should be at least 1 cm) and
filled all cavities and channels without air congestion. Across
certain time intervals, depending on the chemical composition

means (from 5 to 30 minutes), the test product is removed from the solution and
is placed for 5 minutes. in a solution of the corresponding neutralizer,
tested for efficiency. After this, the test items are transferred to
tubes with a liquid nutrient medium (SPB) with 0.5% glucose. Crops
are kept in a thermostat at a temperature of 37 ± 1 ° C for 21 days.
Preliminary results are recorded after 48-72 hours, and
the final - for 21 days. As a control,
test objects, contaminated as described above, and placed in
neutralizer and in tap water for the time of maximum
sterilization exposure.

The regime (concentration-time-temperature) is considered to be effective;
providing 100% death of the test-microbe spores at all
test objects in the absence of it in the neutralizer. In the presence of
positive experiments are repeated, increasing the time
impact, but not more than up to 16 hours. Sterilization mode,
developed on imitators of the endoscope channel,
treatment of an endoscope contaminated with a test microorganism.

The criterion of effectiveness is to achieve 100% death of the dispute
test-microorganism on all test-products. In this case, the action time
solution should not be more than 16 hours at a temperature
18-20 ° C, and no more than 3 hours at a moderately elevated temperature
50 ± 1 ° C.

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1.7.9. Apps

Annex 1 (informative)

Characteristics of test microorganisms according to morphological, cultural, biochemical properties inherent in this species:

1. Bacillus cereus (strain 96).

Movable gram-positive rod; size, micron: length - 3-5, width - 1,2. Growth temperature ° C: minimum - 10-20; optimal - 37 ± 1, the maximum - 35-45. Disputes the size of 0.8 × 1.2 μm, elliptical or cylindrical shape, located centrally. Aerobe. On MPB (pH 7.3 ± 0.1) after 24 hours Incubation forms a crumbly sediment and a parietal ring, forms R-forms; on MPA (pH 7.3 ± 0.1) - round, dry, white, convex colonies with an uneven edge, 3-5 mm in size; products splitting glucose: acid, acetone, gas is not formed. The colorability of vegetative cells according to Gram, the dispute - according to Tsilu-Nilsson. By classification of pathogenic for rights microorganisms belongs to the IV group of pathogenicity. Bacillus cereus are used to study and evaluate the sporicidal activity of DS, sterilizing agents and their substances on the basis of oxygen and of the chlorinated DW.

The test-microorganism of B. cereus is stored in the museum of the Federal budgetary institution of science "Research Institute disinfectology "of Rospotrebnadzor (117246, Moscow, Scientific fare, 18).

2. Geobacillus stearothermophilius (strain VKM B-718).

Mobile gram positive thermophilic wand. The optimum growth temperature is 55 ± 1 ° C, excluding the development of other

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widespread microorganisms. Disputes are elliptical, located centrally. Aerobe. On MPB (pH 7.3 ± 0.1) after 24 hours incubation forms a turbidity of the medium, on MPA (pH 7.3 ± 0.1) - slightly convex colonies 2-4 mm in diameter, with an even edge. Reaction Foges-Proskauer the negative. Fermenting glucose from acid formation; Do not ferment arabinose, xylose and mannin. Hydrolyzes casein. Does not cleave phenylalanine and tyrosine. Non-pathogenic to humans and animals. G. stearothermophilius are used to study and evaluate the action of water-rich steam under excessive pressure during sterilization of medical products.

The test-microorganism G. Stearothermophilius is stored:

- in the Museum of Cultures of the Federal Budgetary State institutions science "Research institute disinfectology "of Rospotrebnadzor (117246, Moscow, Scientific fare, 18);

- Museum of Cultures of the Testing Laboratory Center (SUE) "Moscow City Center for Disinfection" (129337, Moscow, Yaroslavl highway, 9).

3. Bacillus licheniformis (strain G VKM B-1711D).

Mobile gram positive wand. Optimum the growth temperature is 37 ± 1 ° C. Spores elliptical, located centrally. Facultative anaerobic. On the MPB (pH 7.3 ± 0.1) through 24 hours of incubation forms a turbidity of the medium, on the surface - dry film; on MPA (7.3 ± 0.1) - slightly convex dry colonies elongated star-shaped with uneven edges 4-6 mm in diameter, growing into a nutrient medium. The reaction of Foges-Proskauer positive. Ferment glucose with the formation of acid and gas.

Reduces nitrates, forms a gas in a medium for nitrification in anaerobic conditions. Disposes of propynate. Hydrolyses starch. Cleaves arginine. Does not form indole, lecithinase, acid in litmus milk. Non-pathogenic to humans and animals. Vacillus licheniformis is used for the study and evaluation of sporicidal activity of dry hot air during sterilization of medical

products.

The test-microorganism of B. licheniformis is stored in:

- Museum of Cultures of the Federal Budgetary Science Institution "Research institute disinfectology » Rospotrebnadzor (117246, Moscow, Nauchny proezd, 18);

- Museum of Cultures of the Testing Laboratory Center (SUE) "Moscow City Center for Disinfection" (129337, Moscow, Yaroslavl highway, 9).

4. Bacillus subtilis (strain 7).

Movable gram-positive rod with a size of 2-3 × 0.7-0.8 m. The growth temperature, ° C: the minimum - 5-20, the optimum - 37 ± 1; the maximum is 45-55. Disputes measuring 0.8 × 1.2 μm, elliptical or cylindrical shape, are located centrally. Aerobe. On liquid nutrient media forms a film of grayish-white color; on dense The nutrient medium forms the R-form, the folded colony of the corporal colors with cut edges of 3 × 5 mm, easily removable loop. It decomposes glucose into acid, acetone; gas does not form. The colorability of vegetative cells according to Gram, the dispute - according to Tsilu-Nilsson.

Vacillus subtilis is used for the study and evaluation of sporicidal activity of DS, sterilizing agents and their substances on the basis of aldehydelective DV.

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The test-microorganism Vacillus subtilis is stored in the Museum of Cultures Of the Federal budgetary institutions science "The scientific-Research Institute of Disinfectology "of Rospotrebnadzor (117246, Moscow, Scientific passage, 18).

5. Vaccine strain B. anthracis STI-1 (pX01 $^{+}$, pX01 $^{-}$). Strain STI-1 is a mobile gram-positive rod 3-5 × 1.0-1.2 μm. The growth temperature, ° C: the minimum - 15-20, the optimum is 37 ± 1; the maximum is 40. Spores of 0.8 × 1.2 μm in size, elliptical or cylindrical shape, are located centrally. Anaerob, on liquid nutrient media grows in the form of cotton flakes, weighted lumps, does not cause turbidity; on a dense nutritious

medium forms R-forms, grayish-white with a fleecy edge ("lion's mane "), colonies 3-5 mm in size. It decomposes glucose into an acid, acetone; gas does not form; does not decompose arabinose, rhamnose, mannose, galactose, raffinose, lactose, heculin, mannitol, dulcide, sorbitol, inositol; does not have hemolytic, phosphatase, lycisinase activity; lysed specific anthrax phage; gives positive reaction with luminescent adsorbed anthrax serum, sensitive to impact anthrax and penicillin, does not cause hemolysis erythrocytes; when sowing on 5% blood agar, does not coagulate the egg yolk. Used in the study and evaluation of sporicidal activity DS and their substances in the form of an anthrax live STI-1 vaccine for people (MTPU-42 №10) (live spore culture of anthrax vaccine strains).

6. Bacillus anthracis, strain 81/1 (pX01 $^{+}$, pX02 $^{+}$) or 27 (pX01 $^{+}$, pX02 $^{+}$).

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Movable gram positive sticks size 3-5 × 1.0-1.2 μm. The growth temperature, ° C: the minimum - 15-20, the optimum is 37 ± 1; the maximum is 40. Spores of 0.8 × 1.2 μm in size, elliptical or cylindrical shape, are located centrally.
Anaerob, on a liquid nutrient medium, grows in the form of cotton flakes, weighted lumps, does not cause turbidity; on a dense nutritious medium forms R-forms, grayish-white with a fleecy edge ("lion's mane "), colonies 3-5 mm in size. It decomposes glucose into an acid, acetone; gas does not form; does not decompose arabinose, rhamnose, mannose, galactose, rafenose, lactose, heculin, mannitol, dulcide, sorbitol, inositol; does not have hemolytic, phosphatase, lycisinase activity; lysed specific anthrax phage; gives positive reaction with luminescent adsorbed anthrax serum.

Used in the study of sporicidal efficacy DS at disinfection of various objects using biological

method of control.

Annex 2 (recommended)

Method for preparation of nutrient media for cultivation
test micro-organisms for study and evaluation
sporicidal activity of DS, CC and their substances

1. Hottinger agar.

Meat digest by Hottingeru (amine nitrogen from 140 to 160 mg%) - 1000 ml;

Sodium chloride - 5.0 g;

Agar 20.0 g;

pH 7.3 ± 0.1.

Meat perevar on Hottingeru diluted with distilled water to an amine nitrogen content of 140 to 160 mg%, is added chloride sodium and agar, boil on low heat with constant stirring to complete melting of the agar. Filter and sterilize at temperature 120 ° C for 30 min.

2. Wheat agar.

Wheat groats "Artek" (or "Poltava" - 500,0 g;

Agar - 25.0 g;

Distilled water - 1000 ml;

pH 7.3 ± 0.1.

Wheat groats "Artek" (or "Poltava") are poured distilled water. After 18-24 hours, the infusion is gently drained, not

squeezing, bring to the original volume, add agar and
melt in a water bath or in an autoclave (with steam for 1 hour).
Leave in a warm place until precipitation settles for 12 hours. Cooled down
Agar is spread on a baking sheet and the sediment is cut. The agar is melted
in a water bath, stirring constantly. The pH is adjusted to 7.3 ± 0.1.
Poured into bottles, test tubes, mattresses (depending on the solved

tasks and objectives). Sterilize with a flowing steam, in a Koch apparatus, for 1 h
for 3 days.

3. Potato-peptone agar.

Peptone - 5.0 g;

Chalk - 1.0 g;

Agar - 25.0 g;

Potato water - 1000 ml;

pH 7.1 ± 0.1.

Raw potatoes (at the rate of 200 g of peeled potatoes per 1 liter
tap water) are thoroughly washed, peeled and peeled,
cut into small slices, pour tap water and
boiling for 30 minutes. after boiling (use new potatoes
not). The broth is decanted and filtered in a cold state through
cotton-gauze filter. The volume of the filtrate is brought to the original volume.
The pH is adjusted to 7.1 ± 0.1. Peptone and agar are added. Heat,
stirring, until the agar is completely melted, filtered through a cotton-
gauze filter, after which add chalk. Poured into bottles,
sterilized at 120 ° C for 30 minutes. After sterilization, the medium in
flakes mow down.

4. Meat-regulating agar.

The preparation procedure is set out in the "Handbook on
microbiological and virological methods of research "(under
Ed. M. O. Birger - M., Medicine, 1982).

5 . Nutrient agar for the cultivation of microorganisms dry,
NGO "Nutritional Environment", Makhachkala (St. Petersburg).

Nutrient agar for the cultivation of microorganisms is prepared
according to the label on the label.

6. Hottinger broth.

Meat digest by Hottinger (amine nitrogen 140 to 160) mg% -

1000 ml;

Sodium chloride - 5.0 g;

Glucose - 5.0 g;

pH 7.3 ± 0.1.

Meat perevar on Hottingeru diluted with distilled water to an amine nitrogen content of 140 to 160 mg%, is added chloride sodium. Boil on low heat with the lid closed 10 minutes. before complete dissolution of the salt. If there is a boil-off, bring the volume up to initial distilled water. The pH is adjusted to 7.3 ± 0.1, boil repeatedly. Filter, add glucose. Poured into sterile dishes. Sterilized at 110 ° C for 30 minutes.

7. Meat-buying broth.

The preparation procedure is set out in the "Handbook on microbiological and virological methods of research "(under Ed. M. O. Birger - M., Medicine, 1982). For preparation of broth with glucose add 0.5% glucose.

8. Nutrient broth for cultivation of microorganisms dry, NPO "Nutritional Environment", Makhachkala (SPA).

Nutrient broth for cultivation of microorganisms prepare according to the label on the label. For the preparation of broth add glucose (0.5%).

9. Color nutrient medium with bromocresol indicator purple.

Nutrient broth for cultivation of microorganisms dry-prepared according to the label on the label;

Glucose - 5 g;

Bromocresol purple spirit 1% - 2 ml;

Distilled water - up to 1000 ml;

pH 7.3 ± 0.1.

In a nutrient broth for the cultivation of microorganisms dry, prepared according to the label on the label, add 5 g glucose, stirred until complete dissolution of the latter, filtered through a cotton-gauze filter and add 2 ml of 1% alcohol solution indicator of bromocresol purple.

Set the pH (7.3 ± 0.1) and pour into sterile dishes. Sterilized at 110 ° C for 30 minutes.

10. Color nutrient medium with bromothymol indicator blue.

Nutrient broth for cultivation of microorganisms dry - prepare according to the label on the label;

Glucose - 5 g;

Bromothymol blue alcohol 1% - 2 ml;

Distilled water - up to 1000 ml;

pH 7.3 ± 0.1.

In a nutrient broth for the cultivation of microorganisms dry, prepared according to the label on the label, add 5 g glucose, stirred until complete dissolution of the latter, filtered through a cotton-gauze filter and add 2 ml of 1% alcohol solution bromtimol blue indicator light. Set the pH 7.3 ± 0.1 and poured into sterile dishes. Sterilize with temperature 110 ° C for 30 min.

Annex 3 (informative)

Materials and equipment needed to conduct studies of spore suspension of test microorganisms

1. When determining the concentration of spores in a suspension:

thermostat dry-air TC-80M, TU 64-1-2622-75;

bath water;

pipettes with a capacity of 1.0 and 10.0 cm 3 2nd class accuracy,

GOST 20292-74;

Pipetting dispensers (firms Bcoheat, Finland);

chemical test tubes, GOST 10515-63;

Petri dishes, GOST 25336-82;

Laboratory alcohol, GOST 25336-82 or gas burner;

ethyl alcohol, rectification technical, GOST 18300-72;

household hotplate, GOST 14919-83;

medical rubber tube, GOST 3399-76;

spring clips for rubber tubes, TU 64-1-964-79;

syringing rubber, TU 38.106-141-80;

laboratory chemical stands, TU 48-0534-8-87;

cotton wool medical hygroscopic, GOST 5556-81;

pencil on glass, TU 46-22-904-78; -

stopwatch mechanical, GOST 8.423-81;

thermometers, GOST 215-73;

sterile drinking water;

the nutrient media listed in Annex 2.

2. In determining the stability of a dispute against a flowing pair:

Oyl-Mueller apparatus;

thermostat dry-air TS-80M, TU 64-1-2622-75;

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Pipettes of a capacity of 1.0 and 10.0 cm3 of the 2nd class of accuracy,

GOST 20292-74;

chemical test tubes, GOST 10515-63;

Petri dishes, GOST 25336-82;

Laboratory alcohol, GOST 25336-82;

cambric or whitened coarse calico;

ethyl alcohol, rectification technical, GOST 18300-72;

household hotplate, GOST 14919-83;

medical rubber tube, GOST 3399-76;

spring clips for rubber tubes, TU 64-1-964-79;

syringing rubber, TU 38.106-141-80;

laboratory chemical stands, TU 48-0534-8-87;

cotton wool medical hygroscopic, GOST 5556-81;

a pencil on glass, TU 46-22-904-78;

stopwatch mechanical, GOST 8.423-81;

thermometers, GOST 215-73;

distilled water, GOST 6709-72;

meat-peptone broth (MPB), VFS 42-366 VS-92.

3. In determining the stability of the spores to chloramine, peroxide hydrogen, glutaraldehyde:

laboratory bath;

thermostat dry-air TS-80M, TU 64-1-2622-75;

Pipettes of a capacity of 1.0 and 10.0 cm3 of the 2nd class of accuracy, GOST 20292-74;

chemical test tubes, GOST 10515-63;

glasses of chemical 50-100 ml, GOST 10515-63;

Petri dishes, GOST 25336-82;

Laboratory alcohol, GOST 25336-82;

cambric or whitened coarse calico;

ethyl alcohol, rectification technical, GOST 18300-72;

household hotplate, GOST 14919-83 ;.

medical rubber tube, GOST 3399-76;

spring clips for rubber tubes, TU 64-1-964-79;

syringing rubber, TU 38.106-141-80;

laboratory chemical stands, TU 48-0534-8-87;

cotton wool medical hygroscopic, GOST 5556-81;

a pencil on glass, TU 46-22-904-78;

stopwatch mechanical, GOST 8.423-81;

thermometers, GOST 215-73;

distilled water, GOST 6709-72;

meat-peptone broth (MPB), VFS 42-366 VS-92.

chloramine or hydrogen peroxide;

sodium thiosulfate.

1.8 Research methods and criteria for assessing the effectiveness of funds for pre-sterilization cleaning of medical devices

1.8.1 General

Requirements for the means of pre-sterilization cleaning.

Chemicals recommended for pre-sterilization cleaning products for medical purposes must be following requirements:

have a high washing capacity, that is, for a short time at a temperature of not more than plus 50 0 C ensure removal organic (protein, including blood, fat, etc.) and Inorganic contaminants, including drug residues preparations;

do not have a fixing effect on contact with them; means for combining pre-sterilization cleaning and disinfection of products should not lead to fixing effect in the recommended for such treatment modes;

dissolve well in water;

do not lead to profuse foaming.

Do not damage the workpiece;

have a shelf life of at least 1 year;
means relating to electrochemically activated

solutions (anolytes, catholytes), produced by

Have a shelf life of at least 3 days.

Rules for the study of the means of presterilization treatment.

Means intended for pre-sterilization cleaning

medical products, as well as active ingredients for production

Before starting the research of detergent properties,

check for a fixing action in the following cases:

if the composition of the product includes alcohol and / or aldehyde

components, and / or amines, peracids or their derivatives;

if a new active substance is presented for research

or the composition of the tool includes a new component (s), relative to

(s) there is no data on the fixing action.

Studies of powdered products, their working solutions

conduct only if they are completely dissolved in drinking water

(if the developer does not specifically stipulate the possibility

The presence of residues of undissolved components that are not

should / can not have adverse effects on detergents

properties of the tool).

To assess the effectiveness of active substances (substances),

intended for the production of pre-sterilization

cleaning, and also the means of pre-sterilization cleaning in

laboratory conditions as a test contamination is used

donor blood.

1.8.2. Investigation of the effectiveness of active substances (substances),
intended for the production of pre-sterilization
cleaning medical products

Investigation of the fixing properties of active substances.

For definitions fixing actions use

test products of the following materials:

metals (surgical scalpel pointed or

abdominal, dental diamond disc);

rubber from natural and synthetic rubber (fragments drainage pipes 10 mm long);

Glass (slide or Petri dish lid).

On the day of the experiment on the surface of each test-products (always on the surface of the canal of the drainage tube) using eye or glass (volume 1-2 ml) pipette apply two drops (diameter not less than 3 mm) of the canned donated blood and dried until completely dry during 45-90 min. (depending on the humidity in the room).

Prepare working solutions of the active substance with various, subject to concentration studies.

Experiments are conducted at room temperature and the solution temperature of the medium is 20 ± 2 ° C.

Test-products contaminated with blood, after drying immerse (avoiding contact with the edges of the container) in prepared working solution for different time (max. the time of action of the solution should not exceed 20 minutes).

At the end of the exposure time, each test product rinsed under a stream of running (120 l / h [3]) drinking water in for 30 seconds, avoiding the direct hit of a stream of water in the field, originally contaminated with blood.

To evaluate the fixing properties, working solutions concentrations and at the time of exposure Effective in the evaluation of antimicrobial properties. All manipulations is carried out in the same manner as above.

[3] The volume of water is measured using a measuring vessel and a stopwatch. Through The water tap regulates the water flow in such a way that in 30 seconds a measuring vessel came 1 liter of water.

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The presence of fixing properties of active substances is judged, visually assessing the presence of blood residues on the surface test-products in places of drawing of blood after its endurance in working solution of the agent and rinsing under running potable water.

Criteria of fixing action.

Group A – absence of visually observed aureole on surface of the test product in the places of initial application of the drops blood testifies that the solution does not have a fixing actions (symbol " – ").

Group B – presence of visually observed aureole on surface of the test product in the places of initial application of the drops blood testifies that the solution has a weak fixing action (conventional symbol "+").

Group D – τηε presence of visually observable traces of blood on surface of the test product in the places of initial application of the drops blood testifies that the solution has a moderate fixing action (symbol "++").

Group D – presence of visually observed clearly expressed Remains of blood on the surface of the test product in the original application of drops of blood indicates that the solution has a strong fixing action (the symbol "+++").

Outgoing of criteria by fixing action disinfectants should be used for pre-sterilization cleaning, including combined with disinfection, medical products (with dried dirt), if they belong to group A.

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If disinfectants belong to group B, then their can be used for pre-sterilization cleaning, including combined with disinfection, medical products (not allowing drying of impurities).

If disinfectants belong to group D, then their
can be used for pre-sterilization cleaning, including
combined with disinfection, medical products (not allowing
drying) with the preliminary removal of visible
contamination from surfaces and from product channels.

Disinfectants belonging to group D are not
It is advisable to use it for pre-sterilization cleaning, including
number of combined with disinfection medical products.

Determination of the effectiveness of active substances.

To determine the effectiveness (detergent properties) of existing
substances use test products (in an amount not less than 10,
related to a particular group [4]) from various materials: metals
(surgical instruments - tweezers, scalpels, scissors, clamps;
dental instruments - hard alloy and diamond burs,
mirrors, all-metal and with amalgam, tongs, trowels),
glass, plastics, rubber based on natural and silicone
rubber (fragments of tubes 10 mm long and inner diameter
2-7 mm). If it is necessary to determine the effectiveness of the means for
pre-sterilization cleaning medical products other
constructions (endoscopes, instruments to them, etc.) use

[4] specific groups of homogeneous products: – articles having lock parts; – products having channels; – products that do not have locks or channels (except for dental instruments having diamond working parts); – Rotating dental instruments with diamond working parts.

products belonging to a particular group (endoscopes and instruments to
him in an amount not less than 3).

New test products are pre-cleaned of oils,
mechanical impurities, washed and sterilized by a steam method.
Test products from metals and glass can be sterilized
air method.

As test contamination, donor blood is used.

The experiments are carried out at an ambient air temperature of 20 ± 2 ° C.

On the day of the experiment, test products are contaminated

donor blood with eye or glass (volume 1-2 ml)
pipettes:

- on the surface of the working part, test products of metals are applied one drop and in the latch part two drops of blood (the diameter of each drop not less than 3 mm), and do several working movements for penetration of blood in hard-to-reach areas of the castle part tool;

- Inside the test pieces made of rubber, plastics and glass cause two drops of blood to be distributed throughout the internal surface of the test product.

Contaminated test products are dried until completely dry in within 45-90 minutes. (depending on temperature and humidity of air in room).

Prepare working solutions of the active substance with various, subject to concentration studies. Working temperature solution should be within 18-22 ° C.

Test products contaminated with blood are immersed in cooked Working solution for different times (maximum exposure time solution should not exceed 20 minutes), each time after the end

which is carried out by mechanical cleaning with the help of various devices (brushes, ruffs, gauze napkins, cotton swabs, syringes - depending on the design features of the products) in The same portion of the solution in which soaking was carried out, and then rinse under running potable water for 1 min.

When working with active substances intended at the same time for the production of disinfectants for medical products, to evaluate the detergent properties are used working solutions of those concentrations and at the time of exposure that Effective in the evaluation of antimicrobial properties. All manipulations is carried out in the same manner as above except for step washing under running potable water.

When determining the effectiveness of active substances, intended for the production of pre-sterilization cleaning, in addition to studying the dependence of detergent action on

concentration of the active substance and time of action of the solution,
at need explore addiction effectiveness of
on the temperature and pH of the solution.

Evaluation of the detergent properties of the active substance is carried out
visually, assessing the presence or absence of blood
surface of the test product in the places of application of blood after it
aging in the working solution and rinsing under running water
water, based on the criteria of the fixing action.

If the active substance (substance) belongs to group A-G,
then him can use at production means
pre-sterilization cleaning.

If the active substance (substance) is assigned to group D,
this indicates that the active substance does not possess

sufficient detergent properties for use in production
means of pre-sterilization cleaning.

1.8.3 Investigation of the effectiveness of the means for presterilization cleaning medical products

1.8.3.1 Determination of the effectiveness of agents in the laboratory

Study fixing properties means for
pre-sterilization cleaning of medical devices
similar to the study of the fixing properties of active substances
(Section 1.9.2.). If necessary, test solutions with an initial
increased (but not above + 50 ° C) temperature; specific
the temperature is selected in relation to a particular agent.

Determination of the effectiveness of means for pre-sterilization
manual cleaning.

To determine the effectiveness (detergent properties) of the agents for
Pre-sterilization cleaning using test products similar to
specified in clause 1.9.2.

As test contamination, donor blood is used.

The experiments are carried out at an ambient air temperature of 20 ± 2 ° C.

On the day of the experiment, test products are contaminated

donor blood in the same way as in 1.9.2.

Ready-to-use products (not requiring dilution),
are used in experiments in the original form. From the funds in the form
powder or liquid concentrate prepare working solutions with
different concentrations of active
substances (active substances). The temperature of the finished product or
The working solution of the agent should be within 18-22 ° C. When
need to test solutions with an initial increased (but not

above plus 50 ° C) temperature; a specific temperature is selected
with reference to a particular tool.

Test products contaminated with blood are washed under a flowing
drinking water for 30 seconds. and immerse (avoiding
contact with the edges of the container) into the prepared working solution
(solutions) for different times (maximum exposure time
solution should not exceed 30 min.), each time after the termination
which is carried out by mechanical cleaning with the help of various
devices (brushes, ruffs, gauze napkins, cotton swabs,
syringes - depending on the design features of the test-
products) in the same portion of the solution in which the soaking was carried out.

When working with funds intended simultaneously for
disinfection of medical devices, for evaluation of detergent properties
Use working solutions of those concentrations and at that time
Effects that were effective in assessing
antimicrobial properties. All manipulations are carried out similarly,
specified in clause 1.9.2., with the exception of the washing stage for
running water.

In determining the effectiveness of funds intended for
pre-sterilization cleaning, in addition to studying the dependence
The detergent action on the concentration of active substance and time
effects of the solution, if necessary, investigate the dependence
efficiency from temperature.

Evaluation of detergent properties of the agent is carried out by setting
azopiramic test, assessing the presence or absence of blood on the test-

products (after their aging and mechanical cleaning in the working solution and rinsing under running potable water).

The procedure for setting the azopyramic test. The definition of hidden blood with a reagent kit number 1 (amidopyrine in powder, medical) and reagent №2 (hydrochloric acid aniline, cd) "Azopiram".

Preparation of alcohol solution of the reagent "Azopiram".

10 g of reagent # 1 and 0.15 g of reagent # 2 are mixed in a dry, flask (150 ml), add 50 ml of 95% ethanol (GOST 18300-87), thoroughly mixed until completely dissolved and volume of 95% ethyl alcohol to 100 ml.

Note: Alcohol solution "Azopiram" should be stored in closed glass flask with ground glass stopper in the refrigerator. temperature of 4-8 ° C for not more than 2 months or in a dark place at room temperature (not above + 25 ° C) for not more than 1 month. A slight yellowing of the alcohol solution "Azopiram" in the process storage does not reduce its performance.

Preparation of working solution of the "Azopiram" reagent.

Directly before verification quality pre-sterilization cleaning of products, a working solution is prepared, mixing equal volume quantities of a solution of the "Azopiram" reagent and 3% hydrogen peroxide solution.

The suitability of the working solution of the "Azopiram" reagent is checked if necessary: 2-3 drops of this solution are applied to the blood spot. If no later than 1 minute, a purple staining, then turning into a lilac color, the reagent is suitable for Use if staining for 1 min. does not appear, Reagent can not be used.

Investigation of the quality of pre-sterilization cleaning of products.

Working solution of the reagent "Azopiram" is processed
investigated products: they are wiped with tampons dampened with workers
solution or apply a few drops of the working solution to the working
parts of the test pieces with a pipette.

Attention! Products having lock parts are processed
in addition to working parts and lock parts.

In syringes pour 3-4 drops of working solution of the reagent and
several times push the piston to wet the workers
solution of the inner surface of the syringe, especially the
connection of glass with metal, where most often remains blood, a worker
solution in the syringe is left for 0.5-1.0 minutes, after which it is dislodged
on a gauze napkin.

When checking the quality of needle cleaning, the working solution is collected in
A clean, non-corrosive syringe, and, successively changing
needles, pass the working solution through them, squeezing 3-4 drops on
gauze napkin.

The quality of cleaning of catheters or other hollow articles is assessed
by introducing a working solution into the articles with a clean
syringe or pipette. The working solution is left inside the product in
for 0.5-1.0 minutes, after which it is poured on a gauze pad.
The amount of working solution introduced into the product depends on the
of the product.

Control is subjected to 1% of the simultaneously processed articles
one name, but not less than 3-5 units.

Indication of contaminants.

In the presence of traces of blood immediately or no later than
1 min. After contact of the reagent with a contaminated site, a

coloration, at first violet, then quickly, for several
seconds passing into a pink-lilac or brownish.

Staining, which occurred later than 1 min. after

processing of investigated products is not taken into account.

The working solution of the reagent "Azopiram" reveals the presence of hemoglobin, vegetable peroxidase (vegetable residues), oxidants (chloramine, bleach, washing powder with bleach, chrome mixture for processing dishes, etc.) as well as rust (oxides and iron salts) and acids.

Brownish staining is observed when there are objects of rust and chlorine-containing oxidants. In the remaining cases, the color is pink-lilac.

Features of the reaction.

The articles to be tested must have room temperature (preferably not higher than 25 ° C). It is impossible to check the hot products, and also keep the working solution in bright light or high temperature (near heating appliances, etc.).

The working solution of the "Azopiram" reagent should be used in for 1-2 hours. If the stand is longer, it may appear spontaneous pink coloration. At temperatures above 25 ° C the reagent solution turns pink sooner, so it is recommended use within 30-40 minutes.

After checking, regardless of the results, you should delete the remains of the working solution of the "Azopiram" reagent with the investigated products, washing them with water or wiping with a swab moistened with water or alcohol.

When receiving a "positive azopyramide test" (availability hidden blood on products) should be repeated pre-sterilization cleaning of these products.

Precautionary measures.

Reagent # 1 and reagent # 2 and its solutions should be stored in tightly closed containers separately from food, drugs, disinfectants, strong acids and alkalis.

Preparation of alcohol and working solutions of the reagent "Azopiram" is held on a laboratory table in a well ventilated room, preferably in a fume hood, should be avoid the dusting of reagents. When preparing 3% hydrogen peroxide Perhydrol should be used with rubber gloves.

If the reagents №1 and №2, alcohol and working solutions of the reagent "Azopiram", 3% peroxide solution hydrogen or perhydrol, they should be removed with pure cotton wool or gauze (rags) and wash the contact place with water. If the reagents come into contact with the mucous area of contact should be washed abundantly with a large amount of cold water.

Spilled or spilled reagents are removed, and the place where they are were washed, washed or wiped with water-wet swabs or alcohol.

Alcohol and working solutions of the reagent "Azopiram" refer to Combustible solutions, since they contain alcohol. Therefore it is impossible allow contact with open fire and hot surfaces heating devices.

When preparing and using the reagent "Azopiram" follow the safety rules,

specified in the manuals on labor protection of workers health.

Determination of the effectiveness of means for pre-sterilization cleaning by a mechanized method.

Experimental studies to assess the effectiveness of means for pre-sterilization cleaning by mechanized method is carried out in ultrasound units registered in Russian Federation and allowed to be used in medical organizations for cleaning medical products from metals.

To determine the effectiveness (detergent properties) of the agents for Pre-sterilization cleaning uses test products having lock parts, of metals (surgical scissors, clamps hemostatic, coroncings, dental forceps).

As test contamination, donor blood is used.

The experiments are carried out at an air temperature of 20 ± 2 ° C.

On the day of the experiment, test products are contaminated donor blood with eye or glass (volume 1-2 ml) pipettes: one test piece is applied to the surface of the working part drop and in the latch part two drops of blood (the diameter of each drop is not less than 3 mm), and do several working motions for penetration blood in the hard-to-reach area of the tool's locking part.

Contaminated test products are dried until completely dry in within 45-90 minutes. (depending on temperature and humidity of air in room).

Test products contaminated with blood are rinsed under a flowing drinking water for 30 seconds. (when studying the means, intended for combining the cleaning and disinfection process rinsing of test products are not carried out) and place them in

loading basket of an ultrasonic installation laying them out uncovered, providing free access to the working solution facilities. Test products are placed in no more than 3 layers, while Each subsequent layer is positioned with a shift in relation to test products of the previous layer.

The loading basket with test products is immersed in the working an ultrasonic bath with a prepared working solution studied means and subjected to ultrasound action different times (the maximum time of ultrasonic should exceed 15 minutes .; for dental forceps - 20 min.).

After the end of the ultrasonic treatment, a charging remove the test items and place them in a plastic bag. capacity for rinsing with running drinking water (1 min.).

Quality of cleaning products from blood contamination after treatment with a solution of the agent by a mechanized method is evaluated with the aid of an azopyramic test in accordance with the procedure, outlined in clause 5.9.3.1.

1.8.3.2. The definition of the effectiveness of means in practical conditions

Determination of the effectiveness of means for pre-sterilization
manual cleaning.

Determination of the effectiveness of the agent in practical conditions
conducts personnel performing in medical organizations
preparation of medical devices for sterilization.

Medical tests shall be conducted for at least
1 month with daily use of the treatment agent
products.

To determine the effectiveness (detergent properties) of the agents for
pre-sterilization cleaning manually using products,
which must be sterilized before their
use in patients from different materials (metals,
rubber based on natural and synthetic rubber, plastic,
glass).

Ready-to-use products (not requiring dilution),
are used in experiments in the original form. From the funds in the form
powder or liquid concentrate prepare working solutions with
different concentrations of active
substances (active substances). Experiments are conducted at
temperature of working solutions indicated in the program.

The products to be cleaned are washed under a flowing
drinking water for 30 seconds. and immerse (soak) on
different times (indicated in the test program)
solution) each time after which is carried out
mechanical cleaning by means of various devices (brushes,
ruffs, gauze napkins, cotton swabs, syringes - depending on
structural features of test products) in the same portion of the product
(working solution), in which soaking was carried out. After
the end of the mechanical cleaning of the product is rinsed with a flowing
drinking water (time is indicated in the program) and distilled
water.

When working with funds intended simultaneously for

disinfection of medical devices, for evaluation of detergent properties
Use working solutions of those concentrations and, with that, time

Effects that were effective in assessing

antimicrobial properties. All manipulations are carried out similarly,

specified in clause 1.9.2. except for the washing under flow

drinking water.

Evaluation of the effectiveness (detergent properties) of the agents is carried out

by setting an azopyramic test, assessing the presence or

The absence of blood on products that have been cleaned, in accordance with

the methodology set out in clause 1.8.3.1.

The results of medical tests are issued in the form

protocol.

Determination of the effectiveness of means for pre-sterilization

cleaning by a mechanized method.

The effectiveness of the tool in practical conditions is estimated in

accordance with the Program and methodology of medical

tests of a specific detergent or detergent-disinfectant

means in treatment-and-prophylactic establishments on modes,

specified in this program.

The purpose of the medical tests is to assess the regimens,

developed during the studies of the effectiveness of the means

for the purpose of pre-sterilization cleaning, including combined with

process of disinfection, medical products.

Determination of the effectiveness of the agent in practical conditions

conducts personnel performing in medical organizations

preparation of medical devices for sterilization.

Medical tests shall be conducted for at least

1 month with daily use of the treatment agent

products.

Experimental studies to assess the effectiveness of

means for pre-sterilization cleaning by mechanized

method is carried out in ultrasound units registered in

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Russian Federation and allowed to be used in medical-
preventive institutions for cleaning medical products from
metals.

To determine the effectiveness (detergent properties) of the agents for pre-sterilization cleaning mechanized way use products that must be sterilized before their use in patients, from metals.

Ready-to-use products (not requiring dilution), are used in experiments in the original form. From the funds in the form powder or liquid concentrate prepare working solutions with different concentrations of active substances (active substances). Experiments are conducted at temperature of working solutions indicated in the program.

The products to be cleaned are washed under a flowing drinking water for 30 seconds. (when testing a remedy, intended for combining the cleaning and disinfection process no rinsing) and place them in the charging a basket of ultrasonic installation laying them open, providing free access to the working solution of the product. Products placed in no more than 2-3 layers (determined by the Program tests), with each subsequent layer having a shift in relation to the products of the previous layer.

The loading basket with products is immersed in a working bath ultrasonic unit with a prepared working solution studied means and subjected to ultrasound action different times (indicated in the Test Program).

After the end of the ultrasonic treatment, a charging remove the items and place them in a plastic container

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for rinsing with running potable water (determined by Test program).

Evaluation of the effectiveness (detergent properties) of the agents is carried out by setting an azopyramic test, assessing the presence or lack of blood on products, subjected cleaning mechanized way, in accordance with the methodology outlined in clause 1.8.3.1.

The results of medical tests are issued in the form protocol.

1.9. Methods for studying and evaluating antimicrobial activity disinfectants

1 .9.1. Requirements for the tested disinfectant solution facilities

The tested solutions of the disinfectant are made up in the form of concentrate dilution in accordance with the method immediately before the water is checked for standardized stiffness. Each concentrate before use and before preparation test concentrations must be shaken.

For liquid disinfectants, its concentration is indicated in milliliters (ml) in 100 ml of the total volume (volumetric percent), for dry substances or pastes - in the amount of grams (g) in 100 mg of the total volume. If in the form of dry substances or pastes in the measuring flask is weighed at least 1.0 g ± 10 mg and added water. Percentages refer to a concentrated product, but not to the content of its effective substance.

Special instructions from the manufacturer for obtaining an exact concentrations should be described in detail.

When setting efficiency limits for comparability purposes the following concentrations (%) must be observed. Other concentrations may be necessary to establish effectiveness of certain funds.

100%	7.5%	1%	0.0125%
75%	5%	0.75%	0.00625%
50%	4%	0.5%	0.003125%
25%	3%	0.25%	0.0015625%

20%	2.5%	0.1%	(etc. in
15%	2.0%	0.05%	geometric
10%	1.5%	0.025%	progression)

Note: 100% refers to the concentrated product. All others concentrations refer to concentrations in the test, which means that before Depending on the test conditions, the coefficient must be taken into account.

This transfer does not mean that the in principle successive concentration. On the contrary, depending on the product, the concentrations to be tested are to choose in such a way that the limits of efficiency could be registered with accountable CFU. For this, in the tests "In vivo" also requires different exposure times. Depending on the formulation of the product being tested (for example, iodophore, high alcohol concentrations) may be necessary also check and concentrate. In certain methods, as minimum requirement is a certain reduction in CFU in The result of the action of the selected concentration of the product, so these concentrations should be included in the test.

1.9.2. Reagents, nutrient media, test cultures:

- burdening loads (Appendix A.1.8.);

Casein-soybean-peptone agar;

- casein-soy-peptone solution;
- meat-peptone agar;
- malt extract agar;
- broth of malt extract;
- Saburo's environment;
- Saburo glucose medium;
- Saburo glucose broth;
- BCH;
- SCS;
- neutralizing agents (AppendixA.1.7.);
- ethanol 70%;

Propanol-1 60%;

Propanol-2 70%;

- washing liquids (Appendix A.1.5.);

- twice distilled water;

- water of standard hardness;

- sodium chloride;

- potassium soap (Appendix A.1.6.);

- test media (Appendix A.2.);

- test culture:

- Staphylococcus aureus ATTS 6538 (DSM 799);

- Enterococcus faecitum ATCC 6057 (DSM 2146);

- Enterococcus hirae ATCC 10541 (DSM 3320);

- Escherichia coli K12 HCTC10538;

- Escherichia coli ATCC 11229;

- Proteus mirabilis ATCC 14153 (DSM 788);

- Pseudomonas aeruginosa ATCC 15442 (DSM 939);

- Mucobacterium ternae ATCC 15755 (DSM43227);

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- Musobascerium smegmatis sir 7326;

- Musobasterium avium ATCC 15769 (DSM 44157);

- Candida albicans ATCC 10231 (DSM 1386);

- Aspergilla niger ATCC16404 (DSM 1988);

- Trichofiton mentagrophytes A TSS 3533 (DSM 4870).

Of microorganisms, used for verification disinfectants and antiseptics, receive basic Culture, from which the relevant workers are made culture, and of them - used to test the suspension.

For a description of the preparation of nutrient media, see Appendix A.1.4.

1.9.3. Preparation for Testing

1.9.3.1. Preparation of basic crops

Preparation of culture of bacteria (except for mycobacteria).

From a lyophilized bacterial sample strains is being preparedsuspension in accordance with the recommendations in accompanying documentation for this sample.

Directly from the suspension is seeded on casein-

soy-peptone agar (CSA) or meat-peptone agar (MPA) for the purpose
obtaining individual colonies. Sown cultures incubate
for 18-24 hours at 36 ± 1 ° C.

After incubation, the grown culture is used for testing
purity of the strain and to obtain the main culture for the experiment.
The surface of the Petri dish with KSA or MPA is evenly sown
used by the culture. The crops are incubated for 18-24 hours at 36 ± 1 ° C.

On the surface of the CSA (MPA) -culture, 10 ml of protective,
solution for freezing (see Appendix A.1.4.) and cells

stir. The cell suspension is aspirated from the agar surface,
dilute with a protective solution up to 100 ml and incubate for
30 min. at 20 ° C.

1 ml of the suspension is pipetted into
Low-temperature tubes with glass or ceramic
beads. The tubes are shaken to distribute the cellular
suspension on beads. Excess amount of protective solution
aspirate with a pipette. Low-temperature vials store
at a temperature of no higher than 70 ° C not more than 12 months.

To ensure the quality of the main culture is recommended
clean after freezing for a week, and
also at regular intervals.

Preparation of Candida albicans

The order of preparation of the main crop corresponds
the order of preparation of culture of bacteria. As a nutritious
medium using malt extract agar (SEA), broth
malt extract (SEB) or the Saburo environment. Cultures are incubated
at 30 ± 1 ° C for 42-48 hours.

Preparation of mold fungus culture

The lyophilized culture sample is slurried into
in accordance with the recommendations annexed thereto. Two samples
the suspensions are plated in Petri dishes on SEA or Saburo medium.
The cultures are incubated for 7-9 days at 30 ± 1 ° C.

After incubation, one of the cultures is used to confirm purity of the strain. In parallel, working cultures are laid.

On the surface of the grown culture on agar of SEA (Saburo medium) 10 ml of bidistilled water are applied. Conidia separate with a glass spatula and transferred to a solution. The suspension is transferred to

flask Erlenmeyer and gently shake for 1 min. with Glass or ceramic beads (about 10 beads 3-4 mm in size for every 10 ml of the suspension). The suspension is filtered through fiberglass cotton wool and transferred to a glass tube for centrifugation. The filtered suspension is centrifuged 20 minutes. at 2000 rpm, after which a suspension of conidia in 100 ml of a protective solution for freezing (see Annex A.1.4.).

1 ml of the suspension is pipetted into low-temperature bottles with glass or ceramic beads. Tube shake to distribute the cell suspension along the beads. The prepared composition is left for 30 minutes. at 20 ° C. Excess protective solution aspirated from help pipettes. The low-temperature test tubes are placed in a freezer at a temperature of minus 70 ° C and store no more than 12 months.

To ensure the quality of the original cultures, it is recommended check for cleanliness after freezing for a week, and then through regular intervals.

Preparation of culture Thrichofiton mentagrophytes

Procedure for preparing the initial culture corresponds to the procedure for preparation of bacterial cultures, only instead of CSA and DAC as a nutrient medium use the Saburo medium or the Saburo broth. Cultures are incubated at 22 + 1 ° C for 14 days.

Preparation of mycobacterium culture

A lyophilized sample of mycobacteria is suspended in 100 ml of a protective solution (see Appendix A.1.4.) in accordance with recommendations attached to it. The mixture is left for 30 minutes.

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at 20 ° C. The suspension is seeded on Middlebrok agar. After incubation for 21 days at 36 ± 1 ° C, the grown culture is used to check the purity of the strain. For protection from drying, cup is necessary glue or to wrap up in a plastic bag. In parallel, working cultures are laid.

In a low-temperature test tube, transfer 1 ml suspension. The tubes are shaken to evenly distribute cell suspension on glass or ceramic beads. Then the mixture is left for 30 minutes. at 20 ° C. Excess protection solution remove by pipette. Low-temperature test tubes will interfere with the freezer at a temperature not higher than minus 70 ° C and save there no more than 12 months.

To ensure the quality of the original cultures, it is recommended check for cleanliness after freezing for a week, and then through regular intervals.

Preparation of the culture of Vasillus subtilis, Vasillus cereus

Lyophilized sample of Bacillus subtilis, Bacillus cereus is suspended in accordance with the recommendations. Two samples the suspensions are plated in Petri dishes on MPA or CSA medium. Cultures incubate at 30 ± 1 ° C for 8-10 days. After incubation, one of the cultures are used to confirm the purity of the strain. Parallel lay working cultures. The suspension is transferred to test tubes, centrifuge at 2000 rpm, wash at least 4 times. Suspensions The spores in the test tubes are suspended in 100 ml of a protective solution (see Appendix A.1.4.).

In low-temperature test tubes, transfer 1 ml suspension. The tubes are shaken to evenly distribute suspension of spores over glass or ceramic beads. Then the mixture

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leave for 30 minutes. at 20 ° C. Excess protective solution is removed with using a pipette. Low-temperature test tubes interfere with

freezing chamber at a temperature of no higher than minus 70 ° C and retained not more than 12 months.

1.9.3.2. Preparation of working crops

Preparation of culture of bacteria (except for mycobacteria)

In order to prepare a working culture of bacteria, Using tweezers from the original culture, separate beads are taken and are suspended in the DAC (BCH). From this suspension, by sowing KSA (IPA) is laying two subcultures: one of them serves for enrichment, and the other to confirm the purity of the strain. Both subcultures are incubated at 36 ± 1 ° C. After incubation for 18-24 hours from enrichment culture lay the second subculture. If this can not be done in the same day, then for further re-sowing A 48-hour subculture is allowed, provided that this subculture for 48 hours was in the thermostat. In these conditions, the next 24-hour subculture Before continuing the execution of the method. The second and / or third subculture is a working culture. Preparation of the fourth subculture is not allowed.

Preparation of Candida albicans

To prepare a working culture from tubes with initial The culture of the tweezers is used to remove individual beads and suspend them in bidistilled water. From this suspension, by sowing SEA (Saburou environment) are laying two subcultures: one serves for enrichment, the second is designed to produce individual colonies with

further determination of the purity of the strain. Both subcultures incubate at 30 ± 1 ° C.

After incubation for 42-48 hours from the culture, The second subculture is laid for enrichment. If this is impossible on that day, then for further sowing A 72-hour subculture is allowed. Under these conditions It is necessary to prepare the next 48-hour subculture, before

than continue the execution of the method.

The second and third subculture is a working culture.

Preparation of the fourth subculture is not allowed.

Preparation of mold fungus culture

To prepare a working culture of mold fungi from

Vials with the original culture with tweezers are extracted by individual beads

and suspended in bidistilled water. From this suspension

by means of crops on the SEA, two subcultures are laid. One of

they serve for enrichment, and the second for obtaining individual colonies

to confirm the purity of the strain. Both subcultures are incubated

for 7-9 days at 30 ± 1 ° C.

Using a culture intended for enrichment, cells

is suspended in 10 ml of bidistilled water. Conidia separated from

the surface of the culture with a glass spatula. The suspension is transferred to

flask of Erlenmeyer and lightly shaken with glass beads in

for 1 minute. The suspension is filtered through a sintered filter

of glass particles.

Preparation of culture Trichophyton mentagrophytes

To prepare a suspension of Trichophyton mentagrophytes

use cultures on Saburo's medium. After incubating the cultures in

for 2 weeks at 22 ± 1 ° C, each covered with a layer of 10 ml of DAC.

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The colonies are then carefully removed with a loop and transferred to the next 10

ml DAC. To remove larger particles, the suspension is necessary

filter through fiberglass wool.

Preparation of mycobacterium culture

For the preparation of a working culture of mycobacteria, individual

The beads with tweezers are extracted from the tubes with the original culture and

is suspended in bidistilled water. From this suspension c

Using the crops on Middlebrok agar, two subcultures are laid.

One of them serves for enrichment, and the second one is used for

confirmation of the purity of the strain. Both subcultures are incubated

at 36 ± 1 ° C. To protect from drying, the cup must be sealed or

place in plastic bags. After 21 days from the culture,

intended for enrichment, lay the second subculture.
The first and / or second subculture is a working culture.
Preparation of the third subculture is not allowed.

Preparation of the culture of Bacillus subtilis, Bacillus cereus

To prepare a working crop, separate beads
The tweezers are removed from the tubes with the original liquid and
is suspended in bidistilled water. From this suspension c
Two subcultures are laid by the sowing of IPA crops. One of them
serves for enrichment, and the second for obtaining individual colonies for
confirmation of the purity of the strain. Both subcultures are incubated
7-9 days at 30 ± 1 ° C.

Using a culture designed for enrichment, spores
is suspended in 10 ml of bidistilled water. Disputes are separated from
the surface of the culture with a glass spatula, transferred to
centrifuge tubes, centrifuged by washing at least 4 times.

1.9.3.3. Confirming the purity of the strain

The purity check is carried out by sowing the strain on one or
several cups with the appropriate nutrient medium, and
microscopically.

The identity of the strain must be confirmed by
relevant identification methods.

1.9.3.4. Preparation of test suspensions

Preparation of culture of bacteria (except for mycobacteria)

Bacterial or yeast growth on the surface of the nutrient
working medium with a glass spatula
is suspended in 10 ml of a dilution agent
(see Appendix A.1.Z.). Larger particles are removed by precipitation
or filtration through fiberglass wool. Number of CFUs
in a test suspension by suitable methods when used
diluent is adjusted to 1.5×10^9 -$8x10^9$ / ml (from $1.5x10^8$ to $8x10^8$ ml for
yeast). This suspension should be stored at 20 ± 1 ° C and used

within 2 hours. Immediately before the test
The required amount of the suspension must be brought to
temperature at which the test is carried out.

Preparation of mold fungus culture

Conidia separated from the surface of nutrient agar with
using a glass spatula, is suspended in 10 ml of a diluent. This
the suspension is shaken gently for 1 minute in a glass flask
(100 ml) with 5-10 g glass beads 3-4 mm in diameter, and then
filtered.

Through microscopic examination directly
after preparation of the test suspension or shortly before
test, if the suspension is used for more than 4 hours,
absence of fragments of mycelium and germination
dispute. These phenomena should be observed in less than one in ten
counting sites.

Use of a suspension with sprouted spores is not allowed.

In case of detection of mycelium, the filtered suspension
centrifuged for 20 minutes. at 2000 rpm, precipitated and resuspended
is suspended to wash the mycelium with a diluent. This
the process is repeated until the absence
Mycelium, but not less than twice.

The amount of spores in the test suspension is reported
up to (1.5-8.0) × 10^{8} / ml, after which the suspension can be used in
for 2 days at a storage temperature of 2-8 ° C. Before use
The test suspension must be shaken.

Preparation of culture Trichophyton mentagrophytes

The microorganisms grown on Saburo's medium are transferred to
tubes with 10 ml NaCl and glass beads, homogenize in
test tubes. Controlled suspension must contain
from 10^{7} to 10^{8} cfu / ml.

Preparation of mycobacterium culture

Surface growth of the working culture is washed off in 10 ml

bidistilled water, three times centrifuged for 15 minutes. at
2000 rpm. and the precipitate is washed in bidistilled water. After
the last centrifugation of the precipitate is diluted in 5 ml
bidistilled water and homogenized.

The amount of CFU in the suspension should be within
(1.5-8.0) × 10^9 / ml. This suspension, divided into 2 ml portions, with
Storage 2-8 ° C can be used for five days. Before
the test suspension must be shaken.

Preparation of the culture of Bacillus subtilis, Bacillus cereus

Growing on the surface MPA and KSA spore
The microorganisms are suspended with a spatula using 10 ml
(see Appendix A.1.Z.). More
large particles are removed by precipitation or filtration. Suspension
placed in tubes, centrifuged at 2000 rpm. 20 minutes.,
washed at least 4 times. Number of spores of CFU in the test
the suspensions are adjusted to (1.5-8.0) × 10^6 . The suspension is transferred to a vial and
heated in a water bath at 75 ° C for 10 minutes. With help
microscopy must be verified in the absence of vegetative
cells and germination of spores. The suspension can be used during
2 days at a storage temperature of 2-8 ° C.

Determination of the initial number of microorganisms

Prepared test slurries with
of the diluent is adjusted to 10^2 -10^3 cfu / ml. The suspension is homogenized.
0.1 ml of the diluted suspension is taken for the control determination,
are inoculated with a surface method and incubated at 36 ± 1 ° C (Candida
albicans for 42-48 hours, Aspergilla niger, Trichofiton
mentagrophytes, Vasillus subtilis - within 7-9 days; M. terrae -
within 21 days). For protection against drying, nutrient media
should be sealed or placed in plastic bags.

The amount of CFU on each nutrient medium is counted.

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1.9.4. In vitro tests

1.9.4.1 Determination of bacteriostatic and mycostatic effectiveness, as well as the suitability of neutralizing agents

The search for a suitable neutralizing agent is an indispensable condition for all experiments.

Microorganisms used in testing:

- Staphylococcus aureus ATTS 6538 (DSM 799);
- Enterococcus faecitum ATCC 6057 (DSM 2146);
- Enterococcus hirae ATCC 10541 (DSM 3320);
- Escherichia coli K12 HCTC10538;
- Escherichia coli ATCC 11229;
- Proteus mirabilis ATCC 14153 (DSM 788);
- Pseudomonas aeruginosa ATCC 15442 (DSM 939);
- Candida albicans ATCC 10231 (DSM 1386).

Preparation of initial, working and test suspensions described in Section 1.9.3.

Preparation of the test solution of the agent

The preparation of the solution of the tested product is described in section 1.9.1.

Test procedure

In the test tubes, mix 5 ml of the solutions of the test substance in the relations in which they are used in tests close to practical conditions (for example, with a chemical-thermal disinfection of laundry), in water of standard hardness with 5 ml of DAC or DAC + neutralizing agent (see Appendix A.1.7.) Double concentration.

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To determine bacteriostatic efficacy in vials

The microorganisms used in the tests are inoculated in an amount of 0.1 ml test suspension diluted with DAC at a ratio of 1:10. For mycostatic efficacy was tested using 0.1 ml undiluted test suspension of Candida albicans. Accounting the results are performed after 48 hours incubation at 36 ± 1 ° C (Candida albicans at 30 ± 1 ° C - 72 hours).

Evaluation of the results

Comparison of the rates of growth retardation in a series of tests without addition and with the addition of a neutralizing agent, provides information on about its effectiveness. A measure of slowing down the reproduction efficiency (oppressive concentration) is considered the greatest dilution product in the DAC or in the DAC + neutralizing agent, which after 48 hours of incubation so inhibits the growth of microorganisms, which is not the turbidity of the suspension ("+" - corresponds to growth microorganisms; «-» - corresponds to lack of growth microorganisms).

If for of various test microorganisms different neutralizing agents are required, then for each microorganism should use the most appropriate means. The neutralizing agents determined in this way are necessary use in all subsequent tests (in vitro tests and tests in conditions, close to practical ones), with the exception of tests with the use of M. terrae.

1.9.4.2 Determination of bactericidal, fungicidal and sporicidal efficiency in a qualitative experiment with a suspension

Note: this study is necessary when subsequent quantitative experiment with the suspension should Not all microorganisms are tested, but only some The most persistent gram-positive and gram-negative

Microorganisms are those that do not matter when assessing the effectiveness of verified method of disinfection. This method can be useful To facilitate the work in determining the range of effective concentrations.

Microorganisms used in testing

- Staphylococcus aureus ATTS 6538 (DSM 799);
- Enterococcus hirae ATCC 10541 (DSM 3320);
- Escherichia coli ATCC 11229;
- Proteus mirabilis ATCC 14153 (DSM 788);
- Pseudomonas aeruginosa ATCC 15442 (DSM 939);
- Candida albicans ATCC 10231 (DSM 1386);
- Aspergilla niger ATCC16404 (DSM 1988);
- Trichofiton mentagrophytes A TCC 3533 (ДСМ 4870);
- Vasillus subtilis cip 7803 (pcs. 96)
- Vasillus cereus cip 7718 (ATCC 9372).

Preparation of working crops and test suspensions are described in Section 1.10.3.

Preparation of the test solution of the preparation, preparation solution of the tested product are described in Section 1.9.1.

Test procedure

0.1 ml of the test suspension is mixed with 10 ml corresponding, disinfectant solution in water

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standard hardness. After incubation for the necessary time suspension of microorganisms with the test medium again is stirred. Select 0.1 ml and transfer to 10 ml of DAC (with The need for a neutralizing agent, as defined in accordance with subsection 1.10.4). These subcultures are incubated in for 48 hours at 36 ± 1 ° C (Candida albicans at 30 ± 1 ° C - 72 hours, Vasillus subtilis - 7-8 days).

Evaluation of the results

By clouding the broth cultures, growth ("+" - corresponds to the growth of microorganisms; "-" - corresponds to the growth of microorganisms; lack of growth of microorganisms).

After this, it is necessary to register effective and ineffective concentration-time relationships for all test microorganisms.

1.9.4.3. A quantitative suspension method using bacteria (except for mycobacteria) and fungi

Concentrations necessary for conducting the experiment are based on results of qualitative experiments with suspensions.

Microorganisms used in testing:

- Staphylococcus aureus ATTS 6538 (DSM 799);
- Enterococcus hirae ATCC 10541 (DSM 3320);
- Enterococcus faecium ATCC 6057 (DSM 2146);
- Escherichia coli ATCC 11229 (K12 HCTC1053);
- Proteus mirabilis ATCC 14153 (DSM 788);
- Pseudomonas aeruginosa ATCC 15442 (DSM 939);
- Candida albicans ATCC 10231 (DSM 1386).

In tests, at least one Gram-negative and one gram-positive bacterium or Candida albicans. Among gram-negative and gram-positive in each case, the most resistant microorganism should be selected according to results of qualitative experiments with suspensions.

For methods operating at temperatures above 60 ° C choose only Enterococcus faecium.

Preparation of initial and working crops, as well as Test suspensions are described in Section 1.9.3.

The preparation of the solution of the tested product is described in section 1.9.1.

Test procedure

A sample of the disinfectant to be inspected is introduced bacterial or suspension of fungi and is maintained at 20 ± 1 ° C in the flow of different exposures. After the selected or installed multiple times the mixture is immediately neutralized in a suitable way to check the bactericidal activity or

fungicide. In each sample, the number of live
microorganisms and calculate their reduction.

The method of selection is the method of dilution and neutralization,
but only if the appropriate means of neutralization is not
found, the application of the membrane filtration method is allowed.

Tests with a protein load are determined by conditions
use of the tested product.

Test procedure:

a) dilution and neutralization method

When carrying out a test without a protein load, 0.1 ml
The test suspension is well mixed with 9.9 ml of solution

verified means. After the expiration of the necessary exposure
mixture of test solution and microorganisms again carefully
is mixed and 0.5 ml is transferred to 4.5 ml of the agent serving for
(see Appendix A.1.3.) (if necessary with
a certain neutralizing agent). After neutralization in
within 5 minutes ± 10 seconds. prepare the dilution from 10^{-1} to 10^{-3} . Then
seeding with a spatula on the CSA (C. albicans - on SEA) from the mixture with
neutralizer and dilutions of 0.1 or 0.5 ml. From each breeding
make 2 sowing.

For inspection, the appropriate test slurry
instead of the product solution being tested, it is mixed with 9.9 ml of the VSW
(control check 1). After the expiration of the necessary exposure
prepare for breeding and seeding on appropriate nutrient media.

The plates are incubated for 42-48 hours at 36 ± 1 ° C (Candida
albicans at 30 ± 1 ° C - 72 hours). To establish the influence of protein
The quantitative experiment with the suspension is carried out with
verifiable solutions of the product containing 0.03% albumin
or 20% of horse serum (see Appendix A.1.V.), which
add immediately before the start of the experiment. For
comparison between experiments with protein loading and
without it, these studies are conducted in parallels.

To simulate high organic pollution

quantitative experiment is carried out with solutions of the tested
Means that contain 0.3% albumin and 0.3% sheep
erythrocytes (see Appendix A.1.8.), which also add
immediately before the start of the experiment. To assess the impact
This research is also carried out in
parallels.

b) The method of membrane filtration

When carrying out a test without a protein load, 0.1 ml
The test suspension is well mixed with 9.9 ml of solution
tested disinfection facilities. After expiration
necessary exposition mixture verifiable solution
with microorganisms again well mixed, transfer 0.5 ml
two separate membrane filters for filtration
containing 50 ml of washing liquid (see Appendix A. 1.5.) and
then filtered. Do not exceed the time interval
1 min., Removed for transport and filtration. Washing is performed
not less than 150 ml and not more than 500 ml of washing liquid.
The membranes are then transferred to diagnostic nutrient media,
so that there is no air leakage between the membrane and the surface
nutrient medium.

Note: when testing the disinfectant soap, the final
The number of bacterial colonies can not be counted, so
It is necessary to carry out the appropriate dilution of solutions with
using a washing liquid.

To control 0.1 ml of the test suspension is mixed
with 9.9 ml of VSW (control check 1). After the necessary
exposure and the corresponding preliminary: dilution of the sample
with a washing liquid, the suspension is filtered as described above
method.

After the expiration of the exposure, a planting up to 10^{-4} -10^{-5},
and 0.5 ml do not transfer the membrane of the filter.

The plates are incubated for 42-48 hours at 36 ± 1 ° C (Candida
albicans at 30 ± 1 ° C - 72 hours). Experiments with protein load and

by carrying out an increased organic pollution: as well as in dilution and neutralization.

Neutralization control (control check 2) and non-toxicity of neutralizing agent (control check 3)

The neutralization control is carried out as follows: to 1 ml the tested disinfectant taken in the maximum concentration, add 9 ml of neutralizer and after 5 min. ± 10 s add 0.1 ml 10 fold dilution (for Candida albicans 10^{-3}) tested suspension. After 60 minutes. 0.1 ml are inoculated into 2 cups appropriate environment. Also, 0.1 ml is inoculated into 2 cups from dilution of the suspension 10^{-1} to the appropriate nutrient media and incubate in a thermostat at 36 ± 1 ° C for 24-48 hours.

Note: if the experiment proves to be insufficient neutralization, then in reproduction by all the relations concentration of duration of exposure should be control checks are prepared 2.

Monitoring of the toxicity of the neutralizer is carried out, as is the control neutralization, only instead of the solution of the tested product is taken VSW.

Processing Results

Take into account the cups in which the amount of CFU lies within between 15 and 300 and count the number of colonies in the experiment and control. After calculating the arithmetic mean of the duplicating determinations, the reduction factor (RF) is calculated by the formula:

1logRF = 1log (CFU Ko) - 1log (CFU D),

where: CFU Co is the amount of CFU per ml without the effect of the agent;

CFU D is the amount of CFU per ml after exposure to the agent.

The values of CFU in terms of the dilution of control checks 2 and 3 should be contrasted with the values of CFU degrees dilution of the test suspension.

In assessing the effectiveness of disinfectants, the criterion Decontamination efficiency is a reduction in the number of microorganisms not less than 5lg.

1.9.4.4. A quantitative suspension method using mycobacteria

Microorganisms and tools used in testing:

– Mussobastria terrae ATCC 15755 (ДСМ43227);

– Musobasteria smegmatis sir 7326;

– Musobasteria avium ATCC 15769 (DSM 44157).

Preparation of initial, working and test suspensions see section 1.9.3.

The following neutralizing agents are recommended:

a) for phenolic disinfectants - polysorbate-80 - 1%, saponin 3%, lecithin 0.3% in phosphate buffer (pH7);

b) for other disinfectants - polysorbate-80 - 1%, saponin 3%, sodium thiosulfate 0.5%, L-Histidine 0.1% in phosphate buffer (pH7);

Preparation of the solution of the tested product is described in section 1.9.1.

Test procedure

A sample of the means to be tested is connected with mycobacterial suspension and this mixture is kept at 20 ° C. After the selected and fixed exposures, a multiple of mixtures are neutralized by an appropriate method for

verification of existing tuberculosis (M. terrae) or mycobactericidal activity (M. avium). In each sample, the the number of living microorganisms and calculate their factor reduction.

The method of selection is the dilution-neutralization method.

Experiments with an organic load are determined by conditions
the use of the disinfectant being tested.

Test procedure

When carrying out the test without an organic load, 0.1 ml
the test suspension is thoroughly mixed with 9.9 ml of a solution
verified means. After the required duration
the mixture of the tested solution with microorganisms again
suspended, taken up to 0.5 ml and transferred to 4.5 ml
a neutralizing agent.
After neutralization for 5 min ± 10 s. prepare the breeding
solutions (from 10^{-1} to 10^{-3}). Then, 0.1 ml from the neutralizing mixture and
dilutions are sown with a spatula on the corresponding
nutrient media (2 cups per dilution).

To control, the test suspension is not mixed with
solution of the test substance, and with 9.9 ml of the VSW (control
check 1). After the necessary exposure, crops for nutrient
the medium is also carried out, as in the experiment.

To protect from drying the Petri dishes are sealed, or
is placed in a plastic bag and incubated for 21 days
at 36 ± 1 ° C.

Description of the experiment with protein load and elevated
organic pollution see in paras. 1.9.4.3 (procedure for conducting
tests).

Neutralization control (control check 2) and
non-toxicity of neutralizing agent (control check 3)

In quality (control check 2) 1 ml of the tested product
in the maximum concentration used in the experiment
are mixed with 9 ml of neutralizing agent and after neutralization in
within 5 minutes ± 10 seconds. is combined with 0.1 ml of 10^{-4} breeding
test suspension. After incubation for 60 min. from here,
as well as from direct composition, as well as from 10^{-1} dilutions in
neutralizing agent with a spatula, apply 0.1 ml on Middlebrok
agar by the method of duplicated determination.

Note: if the experiment shows insufficient
neutralization, then in reproduction by all the relations

concentration - duration of exposure should be

control checks are prepared 2.

Control check 3 is performed in the same way as the control

Verification 2, but instead of the solution of the tested agent, the HCV is taken.

Processing Results

Take into account the cups in which the amount of CFU lies within

between 15 and 300 and count the number of colonies in the experiment and control.

After calculating the arithmetic mean of the duplicating

determinations, the reduction factor (RF) is calculated by the formula:

1logRF = 1log (CFU Ko) - 1log (CFU D),

Where:

CFU Co is the amount of CFU per ml without the action of the agent;

CFU D is the amount of CFU per ml after exposure to the agent.

The values of CFU in terms of the dilution of control checks

2 and 3 should be contrasted with the values of CFU degrees

dilution of the test suspension.

In assessing the effectiveness of disinfectants, the criterion

Decontamination efficiency is a reduction in the number of

microorganisms not less than 5lg.

1.9.4.5. A qualitative experiment of antimicrobial activity
disinfectants using test carriers (rubber tubes)

Microorganisms used in the experiment:

– Staphylococcus aureus ATTS 6538 (DSM 799);

– Enterococcus hirae ATCC 10541 (DSM 3320);

– Pseudomonas aeruginosa ATCC 15442 (DSM 939);

– Mucosbacterium tiera ATCC 15755 (DSM43227);

– Musobasteriitis avium ATCC 15769 (DSM 44157);

– Candida albicans ATCC 10231 (DSM 1386);

– Vasillus subtilis cip 7803;

– Vasillus cereus cip 7718 (ATCC 9372).

Preparation of starting and working crops is described in section

1.9.3.

Requirements for the drug.

The drug for chemical disinfection of the instrument must cause complete death of test microorganisms on all scraps hose.

Preparation of the test solution of the agent.

The preparation of the solution of the tested product is described in section 1.9.1.

Preparation of test carriers.

Cut previously unused hose 1 cm (Appendix A2) is boiled for 10 minutes. in bidistilled water, after which it is dried.

To contaminate the hose cuttings, cultures are prepared in the DAC (for C.albicans in SEB) which were incubated for 24 hours at 36 ± 1 ° C (for C.albicans - 48 hours at 30 ± 1 ° C, for Youillus subtilis, Vasillus cereus on MPA - 7-9 days at 30 ± 1 ° C). Immediately before the start Experiment to these cultures is mixed defibrinated sheep blood in a final concentration of 20%. The amount of living microorganisms of this test suspension (from 1.5-5x10 ^{8} cfu / ml) is detected by means of a surface culture on KSA (IPA) and, accordingly, СЭА (C.albicans).

Prepared trimming of the hose (3 for each exposure and The microorganism is suspended in suspension, after several set by sterile tweezers vertically on the filter paper and dried for 4 hours at 36 ± 1 ° C.

The order of the experiment.

Cut off the hose, immerse in the solution of the test product and after the selected and fixed exposures are transferred to the nutrient medium solution (if necessary with neutralizing agent) and incubated. Guided by the presence or absence growth in test tubes with cultures, determine the effective and ineffective concentration and exposure ratio.

For each microorganism take 12 pieces of hose,

placed in a cup and poured into 30 ml of the solution of the tested
disinfectant. After 15, 30, 45 and 60 minutes. exposure at 20 ° C, tweezers
three trimmings are removed and individually washed in 10 ml
neutralizer for each cut. Then each piece of hose
is transferred to a culture tube with 10 ml KSB (SCS)
(C.albicans - SEB).

As a control check, infected hose clipping
instead of the solution of the product being tested, the VSW is treated and
after 60 minutes. the effects are examined as described above.

With neutralization control for each microorganism, one
The infected hose cut is wetted with a solution of the tested
product, individually washed in 10 ml of CSF (SCA) (C.albicans in
SEB), if necessary with a neutralizing agent. In each
test tube is added one by one pruning the hose of the infected
corresponding microorganism.

Evaluation of neutralization control is that in all
Control tubes after incubation should be observed growth.
The tubes with cultures are incubated for 7 days at 36 ± 1 ° C
(C.albicans - at 30 ± 1 ° C).

Processing of results.

After incubation, test tubes with cultures are examined for
presence of growth.

1.9.4.6. Methods for testing the antimicrobial activity of agents
for surgical and hygienic treatment of hands

Tests of antimicrobial activity are carried out "in vivo"
estimating the level of activity by the reduction factor, the value of which
shows the difference between the decimal logarithms of the number of microbes
on the skin of the hands of probands before and after treatment.

Method for testing the antimicrobial activity of agents

for surgical treatment of hands.

Stage 1.

The probants wash their hands with warm running water and liquid soap

for 2 minutes, dry hands in air or sterile

napkins. After drying the skin of hands, perform washings with two distal phalanges of the four fingers of each arm in sterile Petri dish with 10 ml of neutralizer. Washing is carried out by carefully rubbing your fingers on the bottom of the Petri dish for 1 minute.

Prepare dilutions of flushing fluid on the physiological solution up to 10^{-4}. 1 ml of each dilution was inoculated on the DAC (MPA) depth method. The crops are incubated for 48 hours at a temperature of 36 ± 1 ° C.

2 stage.

Fingers wash with warm running water to remove neutralizer solution and air dried. Then hand brushes in accordance with the instructions for application of the facility. After this, flushing from the fingers in 10 ml of neutralizer solution.

Prepare dilutions of flushing fluid on the physiological solution up to 10^{-3}. For 1 ml of each dilution and a whole flush The fluids are inoculated on the DAC (MPA) by the deep method. Crops incubate for 48 hours at a temperature of 36 ± 1 ° C.

Stage 3.

Count the number of colonies of microbes before and after treatment processing, determine the CFU / ml and the decimal logarithm of the number surviving microbes.

Calculate the reduction factor (RF) using the formula:

1logRF = 1log (CFU Ko - 1log (CFU K1),

Where:

CFU Co - the amount of CFU ml without the action of the agent;

CFU K1 - the amount of CFU per ml after the action of the agent.

The method of testing the antimicrobial activity of agents for

hygienic treatment of hands.

Stage 1.

The probants wash their hands with warm running water and dry their hands on air or with sterile napkins. Further probants The fingers of hands are tested with Escherichia coli test culture ATCC 11229 by lowering the fingers for 1 min. in a glass with a suspension of test culture on a physiological solution of density 10^9 CFU / ml. Arms dried in air until completely dry. After the skin has dried hands, perform washings with two distal phalanges of four fingers each hand in a sterile Petri dish with 10 ml of neutralizer. Washings spend by careful rubbing your fingers on the bottom of a petri dish for 1 min.

Prepare dilutions of flushing fluid on the physiological solution up to 10^{-6} . 1 ml of each dilution is plated on Endomo's environment. The crops are incubated for 24 hours at a temperature of 36 ± 1 ° C.

2 stage.

Then the hands are treated with a remedy in accordance with instruction on the use of the product. After that, for 1 min. spend washings from fingers of hands in 10 ml of a neutralizer solution.

Prepare dilutions of flushing fluid on the physiological solution up to 10^{-3} . For 1 ml of each dilution and a whole flush the liquid is seeded on Endo's medium. The crops are incubated for 24 hours at temperature 36 ± 1 ° C.

Stage 3.

Count the number of colonies of microbes before and after treatment processing, determine the CFU / ml and the decimal logarithm of the number surviving microbes.

Calculate the reduction factor (RF) using the formula:

1ogRF = 1og (CFU Ko) - 1og (CFU K1),

where: CFU Co - the amount of CFU ml without the effect of the agent;

CFU K1 - the amount of CFU per ml after the action of the agent.

Criteria for evaluation.

The agent is considered effective for surgical treatment if in the "in vivo" experiment the reduction factor is caused by a constant and of the adventitious microflora ≥ 2 log.

The product is considered effective for hygienic treatment if the "in vivo" test causes a reduction factor in the test culture E. coli ≥ 4 log.

Appendix A

A.1. Culture media and reagents.

Before starting the experiment, all media and reagents (water, test solution solution, test suspension, neutralizing agent, etc.) should be on a water bath were brought to the experimental temperature of 20 ° C. Consistency the temperature must be controlled.

When carrying out research, all reagents must be pure for analysis and suitable for microbiological

use. The manufacturer's instructions must be strictly observed
on their use.

A.1.1. Water.

Freshly distilled water is sterilized for 20 minutes at 121 ° C.

A.1.2. Water of standard hardness.

– 17.5 ml of a 10% solution of $CaCl_2 x H_2O$;

– 5 ml of a 10% solution of $MgSO_4 x 7H_2 0$;

– add up to 3300 ml, sterilize with steam at 121 ° C;

– pH - 7.2 ± 0.2 at 20 ° C;

Or:

solution A:

– 19.84 g of $MgCl_2$ (anhydrous);

– 46.24 g of CaCl2 (anhydrous).

Dissolve in distilled water and add to 1000 ml,
Sterilize with steam at 121 ° C for 20 minutes.

solution B:

– 35.02 g of NaHCO3

Dissolve in distilled water and add to 1000 ml,
sterilize by filtration (filter pore size 0.22 μm).

In a sterile flask (1000 ml) to 6 ml of solution A add
not less than 600 ml of sterile purified water, add 8 ml of solution B,
add up to 1000 ml with sterile distilled water.

The final hardness should be 300 mg / kg of CaCO3,
pH = 7 ± 0.2 at 25 ° C. To adjust the pH you need either 1-normal
solution of NaOH or 1-normal solution of HCl. Water for
experiments should be freshly prepared and used
not more than 7 days when stored at a temperature of 2 to 8 ° C.

A.1.3. Means for dilution of solutions.

Peptone from casein, digested with trypsin - 1 g;

NaCl 8.5 g;

Double distilled water - up to 1000 ml.

Dissolve and sterilize with steam for 20 minutes. at 121 ° C.

pH = 6.9 ± 0.2 at 20 ° C

A.1.4. Nutrient media.

Feed solution:

Cattle meat extract - 30 g;

Peptone from casein, digested with trypsin - 5 g;

Water twice distilled - 1000 ml.

Dissolve and sterilize with steam for 20 minutes. at 121 ° C.

pH is 6.9 ± 0.2 at 20 ° C.

Protective solution:

Prepare a nutrient solution and before sterilizing with steam add 150 grams of glycerin per liter.

Middelbrook agar:

Agar Middelbrook - 19 g;

glycerol - 5 ml;

water twice distilled - up to 900 ml.

Dissolve, sterilize with steam at 121 ° C for 20 minutes. and Cool to 50-55 ° C; add 100 ml of nutrient component OADC in sterile conditions and mix.

pH = 6.6 ± 0.2 at 25 ° C.

CSA:

Peptone from casein, tryptic digested - 15 g;

soy peptone, papaic production of soy flour - 5 g;

NaCl-5 g;

agar 15 g;

double distilled water - up to 1000 ml.

Dissolve and within 20 minutes, sterilized with steam at 121 ° C.

pH = 7.2 ± 0.2 at 20 ° C.

DAC:

Peptone from casein, tryptic digested - 15 g;

soy peptone, papaic production of soy flour - 5 g;

NaCl-5 g;

water twice distilled - up to 1000 ml.

Dissolve and sterilize with steam for 20 min. at 121 ° C.

pH 7.2 ± 0.2 at 20 ° C.

SEA:

Malt extract - 30 g;

soy peptone - 3 g;

agar 15 g;

water, twice distilled - up to 1000 ml.

Dissolve and sterilize with steam for 20 min. at 121 ° C.

pH = 5.6 ± 0.2 at 20 ° C.

SES:

Malt extract (technical purity) - 20 g;

Water, twice distilled - up to 1000 ml.

Dissolve and sterilize with steam for 20 min. at 121 ° C.

pH = 5.6 ± 0.2 at 20 ° C.

Saburo, IPA, BCH, SCS - cook according to the recipe instructions for use.

A.1.5. Washing fluids

As washing liquids, all liquids must be taken, which are sterile:

– water;

– means for dilution;

– polysorbate-80 (0.1% by volume in aqueous solution);

– polysorbate-80 (0.5% by volume in aqueous solution);

– polysorbate-80 (0.5% by volume in aqueous solution) plus 0.7 g / l lecithin;

– neutralizing agent;

– Buffer solutions.

A.1.6. Neutralizing agents

For each product to be tested,

The appropriate neutralizing agent, which should be sterile.

Some combinations are given below as an example:

– polysorbate-80 - 30 g / l, saponin - 30 g / l, L-histidine - 1 g / l, lecithin - 3 g / l, sodium thiosulfate - 5 g / l, 0.0025 M phosphate buffer to bring up to 1000 ml;

– polysorbate-80 - 30 g / l, lecithin - 0.3 g / l, L-histidine - 0.1 g / l, CAS0-broth - 30 g / l, water, twice distilled - to bring to 1000 ml;

– polysorbate-80 - 30 g / l, lecithin - 3 g / l, L-histidium - 1 g / l, sodium thiosulfate - 5 g / l, saponin - 30 g / l, water - to bring up to 1000 ml with a dilution solution (A.1.3);

– polysorbate-80 - 30 g / l, lecithin - 3 g / l, L-cysteine - 1 g / l, saponin - 30 g / l, water, twice distilled - to bring up to 1000 ml;

– polysorbate-80 - 30 g / l, lecithin - 3 g / l, L-cysteine - 1 g / l, sodium thiosulfate - 5 g / l, saponin - 30 g / l, water, twice distilled - bring up to 1000 ml;

– beta-cyclodextrin - 10 ml, saponin - 30 g / l, water, twice distilled - bring to 1 000 ml;

– polysorbate-80 - 10 g / l, saponin - 30 g / l, lecithin - 3 g / l, 15 M phosphate buffer - bring to 1000 ml;

– polysorbate-80 - 10 g / l, saponin - 30 g / l, sodium thiosulfate - 5 g / l, L-histidine - 1 g / l, 15 M phosphate buffer - to bring up to 1000 ml:

– phosphate buffer 0.25 mol / l, KH_2PO_4 - 34 g, water twice distilled - bring to 1000 ml.

All neutralizing agents are adjusted to a pH of 7 ± 0.2 and sterilized steam for 20 min. at 121 ° C.

A.1.8. Protein loads

a) Protein load:

0.3 g of albumin from blood serum of cattle (fraction V) is dissolved in 100 ml of the dilution agent (see A.1.3.) And sterile filter (the final concentration of 0.3% albumin).

b) Simulation of increased organic pollution:

3.0 g of albumin from blood serum of cattle (fraction V) are dissolved in 97 ml of the dilution agent (see A 1.3) and sterile filtered (final concentration of albumin 3%).

In conclusion, add 3 ml of the solution of lamb erythrocytes (preparation see below).

The final concentration of lamb erythrocytes is 3%.

20% horse serum.

Preparation mutton erythrocytes: 8 ml fresh Defibrinated sheep blood is centrifuged at 800 rpm. within 10 minutes. Drain the liquid over the precipitate, and dilute the sediment in the same amount of the dilution agent (see A.1.3.).

The process is repeated three times until the liquid over the precipitate becomes colorless. Rinse thus washed red blood cells for preparation of protein loads.

2. Entomological and acarological methods of research and criteria for assessing the effectiveness of disinfestations

2.1. General provisions. Organization of experiments.

2.1.1. General Provisions

When developing and studying the means of medical pest control is necessary consider then, what arthropods, having epidemiological and sanitary-hygienic value, are representatives of two classes (insects and arachnids) are to different detachments and families, living in different in different ecosystems.

Moreover, insects are divided into two large groups: with incomplete transformations and complete transformation. In insects with incomplete transformation of the larva and the nymph are externally similar to Imaginal stage, there is no stage of the pupa, and they live in those same conditions as adults. In insects with complete transformation In addition to the egg and larva stage, there is a pupa stage, and larvae completely different from the adult insect, live and develop in other environment. A typical example of an insect with a complete type transformations are mosquitoes, in which larvae develop in aquatic environment, and adults live on land. In addition, for arthropods inherent as a species sensitivity to various chemical substances and physical factors, and different sensitivity stages of development. Different types of arthropods react differently to chemical stimuli that repel or attract them.

In connection with the above, various as substances (physical, biological factors) killing arthropods or affecting the processes of their growth and development, repelling or

attracting them, and the formulations that are most are suitable for use in various biotopes for the purpose of suppressing representatives of the arthropod type, and a variety of methods for studying active substances (substances) and formulations on their basis (drugs, funds).

The following are the methods used to identify and insecticidal, acaricidal (insecticacicidal), repellent and the controlling properties of chemicals, physical and biological agents and means on their basis.

2.1.2. Organization of experiments

Depending on the need to establish insecticidal, acaricidal or other types of actions in substances that do not have such characteristics, carry out research on a limited The number of laboratory cultures of insects and mites in the depending on the class of chemicals concentration or dose.

If any activity is detected, in-depth research, increasing the number of bioobjects, using a series of concentrations or doses.

When assessing the activity of the substances described, their activity in experiments in parallel with the reference sample of such a The same substances, bioobjects and test methods are chosen in dependence from the target activity.

Evaluation of the activity of drugs (preparations) is carried out on laboratory cultures of arthropods, as well as on natural arthropods in laboratory or full-scale experiments in different modes of application.

2.1.3. Arthropod tests used in experiments

Arthropods, used at experiments, may cultivated in the laboratory or caught in natural biotopes. In addition, model objects can be used, which are easy to cultivate in the laboratory, but the data obtained on these objects can be transferred to other target species. This transfer is possible only after carrying out multiple experiments and obtaining reliably convergent data.

Under laboratory conditions, the following are cultivated:

Class Insect Insect:

– α squad of cockroaches: red cockroaches Blattella germanica L., American cockroaches Periplaneta americana L. (international test-objects) and black cockroaches Blatta orientalis L. In experiments use females before extention of ootecs, males 3-21 days old

ages and larvae of different ages. When studying intestinal
The effects of insecticides in experiments use hungry cockroaches,
which 12 hours before the experiment give only water, but do not give food;

– detachment of semi-beetles: bed bug Cimex lectularius L.
In experiments, males and females of 5-day-old age are used,
fed 3 hours before the experiment on guinea pigs, rabbits or
white mice, eggs 3-5 days old;

– α detachment of lice: clothing lice Pediculus humanus corporis De Geer. AT
Experiments use imago (females, 15-day-old males,
fed not earlier than 1 hour before the experiment), 3rd instar larvae,
eggs of any age, depending on the purpose of the experiment. Clothes
lice are easier to cultivate in the laboratory, they are more
resistant to insecticides than head and pubic, so data,

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received on this object, can be transferred to two other types
lice;

– detachment of Hymenoptera: red house ant
Monomorium pharaonis L. In the experiment, workers are used and
females;

– detachment of fleas: rat flea Xenopsylla cheopis Roth. AT
experiments use males and females of 5-day-old age,
fed on white mice 3 hours before the experiment, larvae of the 2nd and
last ages. This species is most significant, because it is
carrier of the plague pathogen, in addition to the species
sensitivity is similar to other types of fleas. When studying
of the repellent activity of substances use hungry imago fleas
2-3 weeks of age;

– detachment of Diptera:

house flies Musca domestica L. In the experiments use
females and males of 3-5-day-old age, fed milk and sugar
syrup, freshly laid eggs, larvae of different ages and
pupae. Room flies are an international test object,
It is possible to test various substances and means, data,

Flies obtained can be roughly transferred to other species of flies and wasps. When studying the intestinal action of insecticides in experiments use hungry flies, which 12 hours before the experiment give only water, but do not give food;

mosquitoes: Yellow-fever mosquito Aedes aegypti L. - international bio-object; Basement mosquitoes Culex pipiens molestus Forsk; Malarial mosquitoes Anopheles maculipennis Meigen, An. stephensi, An. atroparous. In experiments, females of 5-day age, fed with a solution of sugar in water 3 hours before the experiment,

larvae of the 2nd, 3rd and early 4th instars and pupae developing in tap water dechlorinated. When studying repellent activity of substances using hungry imago mosquito 8-10-day age;

– α group of coleoptera: kozheedy Attagenus smirnovi Zhant. AT experiments use imago and larvae 11-12 days of age. It is a test object for all types of tadpoles;

– detachment of Lepidoptera: moth Tineola bisselliella Humm. AT experiments use butterflies and caterpillars of 28-30-day-old age. Is a test object for all types of moles (fur coat, carpet, furniture).

Class arachnids:

– Parasitiform plaque (Parasitiformes):

Ixodes: Ixodes mites (for example, taiga Ixodes persulcatus P.Sch. and forest I. ricinus L.), Dermacentor, Hyalomma and others. In experiments use active hungry imago, nymphs, larvae. The most resistant to chemical and biotic and abiotic factors of adults;

Argas Ornithodorus papillipes (Bir.) nymphs of III and IV ages, starving no more than 5-7 months; females, males, starving no more than 1 year;

gamas: rat tick Ornythonissus bacoti (Hirst). AT The experiment uses adult ticks.

– squad of acariform mites (Acariformes):

home dust mites: Dermatophagoides pteronyssinus (Trouessart) and D. farinae (Huges). In experiments, imago, larvae are used;

scabies: earworm rabbit mite Psoroptes cuniculi (Hering); model object for man's scabies. In the experiment use females.

Arthropod groups, in experiments with which it is advisable to use individuals from natural populations:

– ants: red house ant Ant Monomorium pharaonis L., black garden ant Lasius niger L. and ant Myrmica rubra L. (working individuals);

– lice: clothes Pediculus humanus corporis De Geer. and head Pediculus humanus capitus lice (adults, larvae and eggs);

– mosquitoes: different kinds of genera Aedes, Culex, Anopheles (larvae, adults);

– Ixodid mites all kinds.

The basic principle of choosing between an insect culture and The natural population of arthropods is consistency the experimental response to the target effectiveness of the agent under real conditions of use. When carrying out full-scale experiments a list of species of arthropods increases.

2.1.4. Principles of preparation of working solutions for carrying out experiments and calculations of the activity of substances and preparative forms

For the experiments it is necessary in dependence from bio-objects and the nature of experiments to select the concentrations of workers solutions. First, prepare a series of 4-5 concentrations in increments of dilution 10. As solvents, acetone should be used, ethyl alcohol or water.

Analytical scales take a sample of a substance or a substance and prepare an initial working solution of a certain concentration (C_{ex}), which can be calculated according to the following formulas:

a) for substances according to the formula 1:

$$C_{ref} = \frac{A \cdot AT}{FROM} \quad (1)$$

Where:

A is the required concentration of DV,% (mg / l, mg / ml);

B - the required amount of solution, ml;

C is the concentration of DV in the substance,% (mg / l, mg / ml).

Example 1: from a substance containing 90% LW, it is necessary prepare 100 ml of a solution with a concentration of 0.3% DV.

With $_{exx}$ = 0.3 × 100: 90 = 0.33 ml (g), i.e., 99.67 ml of solvent add 0.33 ml of the test substance.

Example 2: 50% of the emulsion concentrate must be prepared 0.7% aqueous emulsion.

With $_{exx}$ = 0.7 × 100: 50 = 1.4 mL

b) for the formulation:

In the event that it is necessary to prepare a solution of a given concentration for processing a certain area so that to receive the given dosage, calculation under the formula 2 is made:

$$\mathbf{C_{out} = \frac{SD}{C_{prep} \cdot V} \cdot \%100} \quad (2)$$

Where:

C_{prep} - concentration of DV in the medium,% (mg / l, mg / ml);

From $_{ex}$ - concentration of LW in the initial solution,% (mg / l, mg / ml);

D - given dose, mg / m ^{2} (g / m ^{2});

S - area of the surface to be treated, m ^{2} (cm ^{2}, ha);

V is the volume of the preparation for treating this surface, cm ^{3} (ml, l).

Example: you need to get a dose (D) of 0.1 g / m ^{2} at processing disc of filter paper area (S) 78 cm ^{2} (0.0078 m ^{2}) solution in the volume (V) 1 ml. Concentration of LW in the preparation

(C_{prep}) - 5% (0.05). All indicators are used in one dimension -
grams and square meters or kilograms (liters) and hectares.
The interest is given by a decimal fraction (5% = 0.05).

Calculation:

With $_{ref}$ = (0.1 · 0.0078): (0.05 · 1) · 100% = 1.56%

That is, to obtain the initial (working) solution,
prepare a 1.56% solution of the drug (dilute in a ratio of 1:63).

A series of 10-fold dilutions is prepared from the initial solution.
For this, 9 ml of the solvent are added to 1 ml of the stock solution and
as a result, a second dilution is obtained; to 1 ml of the second dilution
9 ml of solvent are added and a third dilution is obtained, etc.
In our example, we obtain a series of solutions, 1 ml of which on filters
standard size provides a dosage of 0.1 g / m^2, 0.01 g / m^2 ,
0.001 g / m^2, etc.

For a more accurate determination of the mean concentrations
(doses) prepare a series of concentrations (at least 4-5) with a dilution step
2 or less depending on the type of arthropod, type of substance
or means.

Insecto-acaricidal activity is estimated by the percentage of death
Arthropods in experimental variants in comparison with the control one.
As a control, you should use either
untreated arthropods, or treated with a solvent,
used for the preparation of working solutions, or contact
Arthropods with a surface treated with a solvent.

Depending on the organization of the experiment, 3 options are possible
evaluation of insecticacaricidal activity.

The experience in which the arthropod is in contact with the subject
substance applied to the surface (or with topical
application of the substance to the body) (experiment), and in the control - with a solvent or
untreated surface. In this case, the insecticacaricidal
the activity of the substance (Y) in each replication is evaluated
by the formula 3:

$$Y = \frac{B_o}{A_o} \cdot \left[1 - \frac{B_k}{A_k}\right] \cdot \%100 , \quad (3)$$

Where:

A_o and A_k - the initial number of individuals in the experiment and in the control;

B_o and B_k - the number of dead individuals in the experiment and in control.

The final score is the average of all
replicates of experience.

If the death of individuals in the control exceeds 20%, then the experiment is rejected.

To obtain more reliable estimates of the drug study
should be repeated with a more viable culture of arthropods
or change the conditions of experience in the direction of greater comfort
for the object in both the control and in the experiment.

If, for some reason, control is not possible and
insecticidal activity should be judged only by the number of
(number) of individuals before and after the application of the substance, formula
simplify (formula 5):

$$Y = \frac{BT}{A_o}_{o} \cdot \%100 \quad (5)$$

To determine the $DM_{50 (95.99)}$, $SK_{50 (95.99)}$ use the graphic
way calculating these indicators, applying

probit-logarithmic paper. On the abscissa axis, doses are postponed
DV (g / m^2 , μg / g insect mass, mg / individual) or concentration (%) in
consecutive dilutions. On the ordinate axis - %% of death
insects at these doses (concentrations). Between the received
points draw a regression line. To determine the DM_{50} (SC_{50})
horizontal line at 50% before crossing with
line chart. The perpendicular dropped from the point of intersection
the abscissa axis will show the desired value on the scale on this axis. Conducting
The horizontal straight line at other levels and dropping the perpendicular,
it is possible to determine the corresponding other values of the SD (CK),
for example, to determine the LED_{95} horizontal line
At the intersection with the line, the graph is carried out at 95%, and so on.

To determine the weight of one arthropod to the experiment

weigh not less than 10 anesthetized individuals (usually from 10 up to 100 individuals).

Conversion of concentration (%) into a dose (μg / g of arthropod mass)

When using the topical method of application,

formula 6:

$$SD_{50}, \mu g / g = \frac{A \times 10 \times Y \times 1000}{AT}, \quad (6)$$

Where:

A is the concentration of SC_{50},%;

Y is the applied volume, μl;

B - mass of arthropod, mg.

Thus, the transfer of any concentration into a dose is possible.

Recalculation of the values of $DM_{50\,(95.99)}$ ($LD_{50\,(95.99)}$) μg per 1 g of mass is carried out by the formula 7:

$$LD_{)99(50} = \frac{x}{the} \cdot 1000, \quad (7)$$

Where:

x - $LD_{50\,(95.99)}$ per 1 arthropod in mcg;

y is the average mass of 1 arthropod in mg.

The average arithmetic value is calculated by the formula 8:

$$X = \frac{\Sigma V}{n}, \quad (8)$$

Where:

V - death of arthropods in each experiment,%;

Σ is the sum sign;

n is the number of replicates.

The error of the mean value of the insecticidal activity of the agent (and other mean values) is calculated from the known error formula average:

$$m_y = \frac{\delta}{n}; \qquad \delta = \frac{\Sigma V^2 - (\Sigma V)^2 / n}{n-1} \quad (9)$$

Where:

V is the value of the insecticidal activity in each of the

replicates of the experiment,%;

n is the number of replicates;

Σ is the sum sign.

because of small number of replicates error medium in entomotoxicological studies can be very great, especially in the range of about 50%, but when the average efficiency to 100% it is reduced. So, with efficiency drug in three replicates of 70, 80 and 90% error is 7.1%, and at efficiencies of 85, 90 and 95% - 3.5%.

To estimate the degree of reliability of the mean value of X under This number of observations uses formula (10) for counting errors of the average:

$$x = \pm \frac{P \times (100-P)}{n}, \quad (10)$$

Where:

P is the standard deviation;

n is the number of observations.

The value of the standard deviation is obtained from formula:

$$X = \frac{\Sigma x_2}{n - 1} \quad \text{(eleven)}$$

Where:

x_2 - the sum of the squares of the deviation from the mean (variance);

n is the number of observations.

Repellent repellent factor for insects are calculated by the formula 12:

$$\mathbf{CODE} = \frac{\mathbf{A} - \mathbf{AT}}{\mathbf{A}} \cdot \mathbf{\%100}, \quad (12)$$

Where:

A - the number of insects on the untreated surface for a certain period of time;

B is the number of insects on the treated repellent surface for the same period of time.

The repellent factor for ticks counts

by the same formula 12, where: A - the number of mites taken in the experiment; AT - number of mites that have passed through the repellent treated zone.

When testing substances with nature, when about their effectiveness judged by reducing the size of the object on the pilot and control sections, the calculation is carried out according to the formula 13:

$$Y = 100 - \frac{A_t \cdot B_o}{A_o \cdot B_t} \cdot 100, \quad (13)$$

Where:

A_o - the number of objects on the experimental site before processing;

In_o - the number of objects in the control area before processing;

And $_t$ is the number of objects after t days (hours) in the trial plot after treatment;

In_t - the number of objects after t days (hours) in the control after processing.

Suction rate index (ISI) for ixodid ticks is calculated by the formula 14:

$$ISP = \frac{V_k}{V_o}, \quad (14)$$

Where:

V_k - average speed of suction of ticks in the control, min .;

V_o - average speed of sucking of ticks in the experiment, min.

When use of method definitions activity Insecticide pencils, crayons, cockroaches need to calculate the area of 3 concentric bands printed on wrapping paper.

The area of each band is calculated by the formula 15:

$$S = \frac{3.14 \times R^2}{2} \text{ cm}^2 \quad (15)$$

When testing the insecticidal activity of agents in aerosol The concentration (C) of the insecticide in the air is calculated in formula 16:

$$C = \frac{Q \times Z}{V}, \quad (16)$$

Where:

Q is the amount of a mixture released from an aerosol can in chamber, determined by the difference in weight of the package before and after the experiment, r;

Z - the proportion of insecticide in the mixture,% of the active substance, recalculated in mg / g, determined by the composition of the filler;

V - chamber volume, m^3.

The C_{15} values (mg / m^3) Is the concentration that 99% of flies or mosquitoes during the conditionally accepted time - 15 min. (formula 17) and Q_{15}, mg / m3 - the amount of the mixture, released from a balloon, causing the defeat of 99% of insects in 15 min. (formula 18).

$$\text{With}_{15} = \frac{C \times T}{15} \quad (17) \qquad Q_{15} = \frac{C_{15} \times 100}{\text{Mass\% of DW}} \quad (18)$$

The coefficient of protective action ($CPD_{mosquitoes}$) of mosquito remedies is calculated by the formula 19:

$$\mathbf{CCD_{mosquitoes}\ (\%) = \frac{BA}{A} \cdot 100}, \quad (19)$$

Where:

A - number of mosquito bites through the control test tissue for period of testing;

B - number of mosquito bites through the tissue of the test test for the period tests.

The deterrent action factor (FLCD of the fly) of the drugs in chamber is calculated by the formula 20:

$$CODE = \frac{K - O}{N} \times 100, \quad (20)$$

Where:

O - number of insects in the experimental chamber;

K - the number of insects in the control (idle) version;

N is the number of insects in total in the experiment.

Coefficient of Attractive Action (CAD $_{FLY}$) preparations

is calculated by the formula 21:

$$KAD = \frac{About - K}{N} \times 100, \quad (21)$$

Where:

O - number of insects in the experimental chamber;

K - the number of insects in the control (idle) version;

N is the number of insects in total in the experiment.

Time of destruction of 50% of individuals of insects (CT_{50}, min.)

is calculated by the formula 22:

$$KT_{50} = [(KT_{99} - KT_{1}): 2] + KT_{1} \quad (22)$$

The index of feeding cockroaches with poisoned baits (IP)

is calculated by the formula 22:

$$PI = \frac{AB}{A + B}, \quad (23)$$

Where:

A - absorption of poisoned bait, mg;

B - absorption of alternative food, mg.

Calculation of the duration of action of the antimolvent at

processing of fabric or fur is calculated according to "Temporary

instructions I-42-2-82 on the work to determine

medicines on the basis of the accelerated

aging "according to formulas 24, 25 and 26:

$$C = K \times Se, \quad (24)$$

Where:

C - expiration date;

C_e is the experimental expiration date;

K - coefficient of correspondence.

$$K = A^{x}, \quad (25)$$

$$x = (T_e - T_{xp}) / 10 \quad (26)$$

Where:

A - temperature coefficient of chemical reaction rate;

T_e is the elevated temperature of 40 ° C;

T_{xp} - storage temperature equal to 20 ° C.

The above dependence is based on the Van't Hoff rule

about a 2-4 fold increase in the rates of chemical reactions with increasing

temperature by 10 ° C. Since in this manual the temperature

the reaction rate of chemical reaction A is assumed to be 2, then in

In our case, x = 2, K = 2x2 = 4. Thus, if we preserve

insecticide for 1 month storage of samples in a thermostat

at 40 ° C, the shelf life of the insecticide at 20 ° C is 1 month. × 4 =

4 months, 2 months. × 4 = 8 months, 3 months. × 4 = 12 months

2.2. Methods for determining the activity of active substances (substances) of disinsection means

2.2.1. General methods for determination of insecticocaricidal properties active substances (substances)

Method topical drawing insectoctacaricide on

Arthropod solutions Insectoacaricide solutions (alcohol or

acetone) of a certain volume with a microdoser are applied

on the mid-flew flies, the mid-breast of bedbugs and cockroaches, or on the dorsal

shield of ixodid or argasid mites, As a microdosage

You can use microsyringes, special applicators or

loops of non-corroding wire. The size of the drop must be

comparable to the size of an arthropod, but should not exceed it.

The following droplet sizes are recommended: for flies, cockroaches and

bedbugs - 1 µL, for bed bugs - 0.5 µl; for ixodochid

ticks - 0.3 µl. In the experiment, not less than 5-7 concentrations

insecticides. At the same time, two control versions are put, in the first

Arthropods are applied a solvent without an insecticocaricide, in the second -

Arthropods are left without treatment. All experiments put

in 3 replicates, in each of 10-20 individuals. During the

experiments, the air temperature in the room should be 21-23 ° C,

the relative humidity is 55-60%.

Arthropods after application of an insecticaricide (solvent) and its drying is placed in clean vessels: bedbugs and argas Mites - in test tubes with filter paper folded "Accordion"; iksodovyh mites - in test tubes with a differentiated humidity; flies and cockroaches - in glasses. Cockroaches give food. Vessels with arthropods are placed under conditions of constant temperature (22 ± 2 ° C) and illumination or at room temperature and natural illumination. Taking into account the results of experiments for room flies spend in 24 hours; Bugs, cockroaches, mites - daily, for 1-5 days depending on the chemical affiliation an insecticaricide. Live animals include individuals capable of directed movement, and individuals, immovable or with sharp violations coordination - to the dead. If mortality arthropods in the control variant is more than 5%, then in the calculations of death it is necessary to introduce an amendment according to the Abbot formula (formula 4).

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At carrying out of experiments use not less than 5 concentrations that should cause death in the range of 0.1-99.9%. The results obtained are processed by probit analysis, building a regression curve "concentration-death" or "dose-death." By The regression curve calculates the parameters of SC_{50} ,% (LD_{50} , μg / g), SC_{95} ,% (LD_{95} , μg / g), SC_{99} ,% (LD_{99} , μg / g).

When specifying data on lethal concentrations or doses it is necessary to indicate the type of arthropod, if necessary the sex, the stage of development, the origin of the race or population, the temperature at conducting experiments. Availability of such information allows compare the data of different experimenters.

If the range is unknown before the start of the study concentrations that cause such death, it is advisable to put An indicative experiment with an interval of concentrations dilution in 10 times.

A precisely dosed amount of solutions of insecticacaricides, as mentioned above, can also be applied to arthropods with using a loop of wire.

Preparing the loop.

The loop must be made of non-corroding material (platinum, rhodium, spiral holder in the light bulb) by a single curl is wound on the needle. The shape of the loop must be round. Then, at the junction of the loop and the rod, a right angle, and the hinge pin is attached to the holder (microbiological holder, collet pencil, etc.).

Loop calibration.

The loop is calibrated by transferring 0.5 ml of alcohol from a small a 1 ml glass beaker onto the filter paper with counting

number of drops transferred. The experiment is repeated at least 3 times. The loop volume is 1 µl (0.5 ml = 500 drops).

The method of spraying insects and test surfaces with sprayers of various types (spray guns, non-propellant packaging, quasars).

Arthropods and test surfaces are placed on a filter paper and sprayed with working solutions, selecting the necessary volume of liquid depending on the object and the test surface. When the arthropods need to be pre-anesthetized. After Drying of the arthropod liquid is transferred to pure plastic or glass beakers.

The method of spraying insects and test surfaces with sprayer with a rotating table (Potter sprayer).

The sprayer consists of the following main parts: liquid atomizer, rotating table for accommodation biological objects, hood, blower with motor for feeding compressed air. Actually the sprayer is made of brass, stainless steel or bronze. The atomizer is inserted into the hole hood, air hose coming from the blower, connect with spray. The hood is usually made of glass or stainless steel with a window for observing the course of spraying, its height 40-45 cm. The sprayer table is made rotating at a speed of 30-40 rpm. to ensure uniform coverage of the surface

drops of insecticide. The table is equipped with a plate with a hole in the middle and sides. On the sprayer table, place cups with immobilized diethyl ether by insects (or unfolded them on a circle of filter paper), put the cap on. Installed on cap sprayer, fill the metered nozzle into the nozzle of the nebulizer

amount of working solution of a certain concentration of insecticide (for example 2.5 ml), include a table motor and an air blower. Liquid flows through the central tube and is broken by a conical jet air passing through the slits between the central tube and tipped. Small drops settle on the table, the walls of the hood and sprayable items. Solutions can be aqueous, alcohol-water or prepared on 60% acetone, since the solutions on pure alcohol or acetone evaporates too much. amount liquid, poured into the sprayer, is selected experimentally, by comparing the results obtained by spraying with results obtained by topical application insecticide calibrated loop or a micro-syringe (with a microdoser).

Methods compulsory contact information arthropods from surfaces treated with insecticacaricides. Arthropods are placed on the surface using two types of without their application.

In the first case use:

- glass exposures Nabokov-Laryukhin height 13-15 cm, diameter 3.5-4.0 cm;

- glass exposures of 8-10 cm in height, 8-9 cm in diameter by the method of CSMA (Chemical Specialities Manufacturers Association), which is used in international practice for testing insecticides.

The contact time in the exposures of Nabokov-Laryukhin is 5-15 minutes, in large CSMA exposures - 1 hour or more.

Exposometers are adapted to work with different insects. Thus, for experiments with cockroaches, extermometers

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smeared with petroleum jelly; for flies - use a special glass
a piston pressing insects to the surface; for fleas -
The exposure meter is covered with a moist multilayer gauze cloth.
Time of contact of cockroaches and bed bugs - 15 min., Rat
fleas and houseflies - 5 min. Insects after contact with
The treated plates are transferred to clean plastic or
glass glasses and record their condition (without external
signs of paralysis, paralyzed, dead) within 24-48 hours.

Contacting imago mosquitoes can also be carried out
in plastic exposition-cones recommended by WHO
for biotests with insecticide treated surfaces.
The internal diameter of the cone is 85 mm at the base, the height is 55 mm.
Cones are made of transparent smooth plastic. In the hole in
center of the cone, mosquito females are triggered using a standard
aspirator with a bent end (diameter 10 mm). Mosquitoes remain in the
exposure (i.e., forcibly contacting the treated
surface) for 30 min. After this, mosquitoes are transferred to
clean cages.

For surface treatment of non-absorbent plates
(glass), use 50 ml / m^2 of the working fluid; for processing
surfaces absorbing liquid (plywood) - 100 ml / m^2

Room flies.

Forced contact of houseflies with active
substances of insecticides are carried out in biological test tubes.
The tubes are treated with acetone solutions of insecticides DV in
logarithmically decreasing concentrations (no less than 5
concentration) at the rate of 1 ml / dm^2 , dried in a warm stream
Air with continuous rotation in a horizontal position.

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Fly contact time is 15, 30, 60 minutes, after which their

planted in plastic containers of about 250 ml
(plastic bottles with cut and tightened gauze bottom).
Insects are given cotton swabs moistened with water. Defeat flies
are taken into account every hour after exposure to 6 hours and
After 24 and 48 hours, the number of dead insects is counted.
Repetition of the experiment 3-5 times in 10 individuals in each variant.

Fleas.

Detection of toxicity of insecticides for insecticides for rat fleas
Xenopsylla cheopis (Roths.) Is carried out using non-absorbed
fleas 1-3 weeks of age without separation by sex, by replanting
on insecticide-impregnated filter paper.

Preparation of impregnated paper leads directly
Before carrying out the experiments by impregnating standard de-impregnated
filters (blue tape) with acetone solutions of insecticides in
logarithmically decreasing concentrations. Flow rate
is 1 ml / 100 cm_2 . After drying, the paper is cut
scissors on rectangles measuring 5 × 1 cm. Impregnated
Paper is used for 3 days, but not earlier than 1 hour after
drying of the solvent.

Adult fleas of laboratory culture are placed in test tubes
20 pieces, then they drop pieces of impregnated paper into them.
After 1 hour, the paper is carefully removed, and test tubes with fleas
is placed in a dark thermostat at a temperature of 26 ° C and a relative
humidity> 50%. Mortality accounting is carried out 24 hours after
end of exposure. Lying fleas unable to independently
turn over, refer to the dead and determine the indicators of SK_{50} ,%
(SC_{95} ,%). With each concentration of insecticide 3 experiments are carried out

not less than 3 replicates, using at least 20 insects per
one repetition. For each experiment, put three control options
(1 - untreated paper, 2 - acetone-treated paper,
3 - fleas in test tubes without paper), in each of which use
not less than 20 insects. From the control options as well as from
Experimental paper is removed after 1 hour.

Ixodid mites.
Contacting ticks from processed

The surfaces are made using filter paper. Paper in
a circle with a diameter of 10 cm (area - 78 cm^2) is placed
horizontally on a non-absorbent surface (glass, glazed
ceramic tiles, etc.) and are pipetted uniformly
on it a solution of the substance under study in acetone (a series of concentrations
not less than 5) at the rate of 1 ml of solution per 100 cm^2 or 0.78 ml per circle.
In the control variant, the same paper is applied to the same circles
solvent method. After evaporation of the solvent, the circles are placed
To the bottom of Petri dishes so that the edges of the circle rise to the walls
cups. Duration of contact of mites with paper 1 min. Mites,
creeping out of the circle, are returned to the paper with a brush.
Since the tongs are sufficiently mobile, simultaneously in a petri dish
it is advisable to put no more than 2-3 mites. In the experience with each
The concentration of the substance used for 30 ticks. Experiments with females and
Male mites are set and taken separately, since there are data
about different sensitivity to acaricides of individuals of different sexes. At once
after contact with ticks the brush is transferred into test tubes
differentiated humidity (10 in vitro), which
placed horizontally under conditions of constant temperature
(about 25 ° C) and illumination or at room temperature and

natural illumination. All work with control tongs
should be held on a separate table using
Uncontaminated tools (scissors, brushes, etc.). Works
with different concentrations it is necessary to start with smaller ones. Accounting
the death of ixodid ticks is carried out one day after the experiment. To the living
include individuals capable of movement, but immobile, almost
stationary and ticks with severe coordination disorders include
to the category of the dead. If the mortality of ticks in the control is
more than 5%, then in further calculations it is necessary to introduce an amendment to the
Abbot's formula (formula 4). The obtained data are processed with
using the probit-analysis method.

The method of free contact of arthropods with surfaces,

treated with insecticacaricides. This method allows more
fully assess the insecticocaricidal agent, taking into account the degree of its
potential repellency.

Cockroaches. Experiments with cockroaches are carried out in special
polygons with an area of at least 0.2 m^2 40×50 cm and the height of the boards
12-20 cm, the edges of which are oiled with petroleum jelly. In the center of the landfill
put a plate (glass, plywood) measuring 10×10 cm, the top
whose side is treated with an insecticide in a certain selected
dose, providing 50, 95 or 99% of the death of cockroaches in the experiment with
forced contact. At the corners of this plate have
Layers in height of 15 mm and from above cover pure plywood
plates of the same size. Thus, a shelter is obtained for
cockroaches.

In each polygon, 120 cockroaches are placed at a ratio
females, males and larvae of II-IV age - 1: 1: 4. In the testing range place
vessels with water and food (white bread). Experience is not less than

in 3 replicates. The account of the dead insects is carried out every 24 hours in
for 3-5 days or more, depending on the purpose of the experiment and
studied means.

Ants.

Experiments with working individuals of ants are carried out in polygons
an area of not less than 0.2 m^2 with a height of boards of 12-20 cm, the edges of which
lubricated with a layer of Vaseline of at least 2 cm wide. In the center of the landfill
put a plate (glass, plywood) measuring 10×10 cm, the top
whose side is treated with an insecticide.

In each polygon place a sheet of paper in the form of loose
a rolled tubule (a refuge for ants), put a drinker with cotton,
impregnated with water, and feed (including white bread, boiled egg,
boiled meat, fish). Then in each polygon 50-100 workers are launched
individuals of ants. Experiments are performed in at least 3 replicates. Accounting
dead insects spend every 24 hours for 2 days.

Flies.

Experiments on flies are carried out in glass chambers with a volume of 1 m^3 ,

having tightened gauze "windows" for ventilation. In each
the chamber is placed on 300 individuals of flies of both sexes in a ratio of 1: 1 and
a vessel with 10% sugar syrup. The camera is illuminated from the outside
electric lamp (60 W). Tapes or plates impregnated
insecticide, in different doses, providing 50, 95 and 99% mortality
flies in experiments with forced contacting, suspended in
the top of the camera. Each variant of experience and control is not
less than 3 replicates. Accounting for lost insects is carried out through
24 hours. The effectiveness of the drug in each version of the experiment
is estimated by the formula 3.

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The method of studying the irritability of mosquitoes under the action of insecticides. Irritability of mosquitoes can be caused not only
insecticide, but also other components that make up the
tested form. Irritability is extremely important when working with
epidemiologically dangerous malarial mosquitoes, because
facilitates the flight of mosquitoes from the treated premises (crib,
outbuildings) in unprocessed (living quarters). Risk
malaria infection is multiplied.

The filter paper is processed from a sprayer with
fine-dispersed head with a working insecticide solution in
concentration, selected in the primary experiment. Research
1-2 days after treatment.

The apparatus for determining the irritability is
opaque camera of lung wood size
135 × 135 × 90 mm. Light enters through the round hole in the back
wall with a diameter of 90 mm, on the sides of which there are grooves for a matte
glass, insecticide-treated paper (or control) and
cone (the same cones as for determining
duration of action of the insecticide). Experiments are conducted in a dark
room with a single light bulb, placed in front of the
the back of the instrument at a distance that depends on the lamp power:
40 W - 41 cm, 60 W - 55 cm, 100 W - 92 cm. Mosquito in the cage in front of

experience in the same room with the same lighting (i.e.
distance from the lamp) for 0.5-1 hour. Every mosquito (well fed)
females) are first put in a control exposition (the same paper, but
without insecticide), where it is held for 3 minutes, and then for 10 minutes.
count the number of takeoffs. Mosquitoes that took off in control 2 or more
once, are rejected as spontaneously irritable. Usually such are few.

The cause of spontaneous irritability can be a mosquito
transplantation or unfavorable conditions (with mass
irritability). Quiet mosquitoes (no ups or 1 random
takeoff without crawling on paper) one by one is transplanted into an experienced
Exposometer with paper treated with an insecticide, and after 3 minutes.
begin counting ups, lasting 10 minutes. Also note
"Walking" mosquito on paper, which also speaks of irritability.
At least 50 specimens should be used in the experiment. At the end
Experiments calculate the distribution of individuals according to the degree of irritability.
For basement mosquitoes Cx. pipiens experiments on irritability also
are important, because when the treatment (with flaws) of mosquitoes,
Those who have irritability choose the untreated
site insecticide.

2.2.2. Methods for studying the fumigation effect of insecticides

Method for studying the fumigation activity of volatile compounds

The experiments are carried out in 1-liter vessels. On the walls of the vessels inside
a filter paper of 10 × 20 cm is placed in advance
impregnated with 2 ml of an acetone (alcoholic) solution of the preparation and
dried at room temperature for at least 20 minutes. When
use of vessels of a larger volume paper size respectively
increase. The calculation of DV is carried out in g / m_3 according to DV. To the lid of the vessels
Suspend the tank from the metal mesh at a distance of 10 cm from the bottom.
In the garden put indoor flies, cockroaches or bed bugs
(not less than 10 insects). In the experiments, a series of concentrations
in 3 replicates. Calculation of dead insects is carried out through
3-6-24 hours. The calculation of the efficiency is carried out according to the formula 5. Determine

The concentration that provides 50, 95 and 99% of insect death.

Method for studying the fumigation activity of volatile compounds for indoor flies. The filter paper is impregnated acetone solutions of insecticides at a rate of 1 ml / dm^2 . Use several concentrations in 2-5 steps. The paper is placed in the middle part of glass vessels with a volume of 10 liters. For comparison Fumigation ability and insecticide of different DW area of the test samples should be the same. In the vessels are placed Insect drinkers (small plastic container with wet cotton wool). Houseflies (imago 3-6-day-old age, without separation into half 100-150 pcs.) are discharged into the vessels, they are closed with a ligature, and fix the time of onset of paralysis with an interval of 5-15 minutes. at for 1 hour and further every 30 minutes. within 6-7 hours. After lesions 95% of insects in the experiment, the paper is extracted from the vessel and through 24 hours account for the proportion of survivors. Evaluation of fumigation activity of insecticides is carried out by comparison indicators - KT50 and KT95 - the time (minutes or hours) for which 50% (95%) of the insects in the experiment are in a state of knockdown and the index characterizing the reversibility of the state of paralysis - the proportion surviving individuals when recorded in 24 hours. Repeatability experiments three times. Experiments are carried out at a constant temperature air of 23-25 ° C. CT_{50} , min. is calculated by the formula 22, or determined graphically.

2.2.3. Methods for studying the intestinal action of insecticides

Method of group feeding of flies with liquid baits.

A solution of the insecticide in 10% sugar syrup (50 ml) impregnate pieces of cotton wool weighing 1 g, which are placed thin layer on the bottom of Petri dishes. When studying substances that are insoluble in

water, 10% alcohol solution is prepared, and then diluted with sugar syrup to the desired concentration. Tests of insecticides are carried out in concentrations of 0.001; 0.01; 0.1; 1.0%, in each not less than 3 replicates. In a gauze cage in the size (20 × 20 × 20) cm, place a petri dish with tested substance (poison bait), as alternative food put a vessel with milk.

Method of group feeding of flies with dry sugar baits.

In the experiments, hungry houseflies were used without separation on the floor of a 3-4-day-old age of sensitive laboratory culture. During the first 3 days after the start of departure, flies as feed give only sugar and water. 16 hours before the experiment, flies they take away the food, leaving only water in the cages. Prepare acetone solutions of insecticide DV, treated with Sugar at the rate of 1 part of the acetone solution in 2 parts of granulated sugar. Sugar sand hangs 2 grams on the watch glass or glass Petri dishes, then pour 1 ml acetone solution of insecticide and leave under traction until complete evaporation of the solvent. To obtain the required dose insecticide in sugar, the initial acetone solution insecticide, given that when applying 1 ml of a 1% solution, that is 10 mg of insecticide DV in 1 ml of acetone per 2 g of sugar, the dose of insecticide in bait is 5 mg / g sugar.

As capacity for flies, you can use or gauze cages in the size 30 × 30 × 30 cm, or transparent plastic containers (bottles) of 2 liters. In the latter case, y bottles cut the bottom and cover it with gauze napkins. Napkins are fixed with rubber rings. The bottle is put horizontally, a cotton swab moistened in the neck

water, in the middle of the bottle is placed the sugar bait on the substrate (small Petri dishes, etc.).

To isolate the intestinal action of the insecticide, carry out in two versions: first - open bait in a cup Petri, and the second - a bait in a fine mesh, to prevent contact the legs of flies with poisoned sugar. In this modification

group feeding is convenient to use instant
refined, which is impregnated with acetone solutions of DV
insecticides, given the mass of each dice. The cubes are placed in
Petri dishes 4 cm in diameter and put in a mesh envelope from
tulle (a fine mesh net), which is tightened and
fasten. The data obtained as a result of experiments with
open and diced cubes, compared according to indicators of SD_{50}
and SD_{95} .

In the experiment, pre-anesthetized with ether
or chilled in the fridge flies. In the control version
use of sugar sand treated with acetone without insecticide in
the same volume as in the experimental version. 3 experiments are conducted no less,
than in 3 replicates, using at least 120 insects per
concentration (40 flies per plastic bottle), in the control
not less than 40 insects are used.

Experiments are carried out at a constant air temperature
(the optimum is 23 ± 2 ° C) and relative humidity of more than 50%.
Allowance for the death of insects is carried out after 24 and 48 hours. Flies lying on
back, unable to turn over and crawl themselves, include
to the dead.

When studying the activity of finished dry sugar bait
(40-60% of sugar) it is applied to plates of glass or plywood, which

is placed in a cage. In experiments, 30 flies of both sexes
3-5 days of age. 12 hours before the experiment in cages, in which
grow flies, leave only water. Controlled by flies,
feeding on milk. Calculation of dead flies is carried out through
3, 24 and 48 hours. The effectiveness of the insecticide is determined by the number of flies,
killed in experimental and control cages (formula 3). Determine
Concentrations that provide 50, 95 or 99% mortality of flies.

Method of group feeding of cockroaches. Experiments with group
the feeding of cockroaches can be carried out in polygons with an area of 0.2 m^2
40 × 50 cm and the height of the boards is 15 cm. On the perimeter of the board,
Vaseline to prevent the care of insects. The polygon is placed

shelter from cardboard, drinking bowl with water, bait and, if necessary,
alternative feed. As a basis for cooking bait
can use dry dog food or extruded
Feed for laboratory animals (rodents). In this case
The granules of the feed are ground on a coffee grinder,
powder in glass bunks of 2 grams, add raw yolk of chicken
eggs for 1 g, acetone solutions of insecticides in a logarithmic way
decreasing concentrations of 0.5 ml. The resulting mass carefully
mix and hang on small petri dishes with a diameter of 4
cm for 1 g. The mass of each bait is written down and the cups are set to dry
at room temperature until completely dry (purchase
constant mass) (about 7 days). Alternative feed is prepared
similar to the control variant, adding instead of an insecticide
the same volume of solvent (acetone).

At least 20 specimens of females are placed in each polygon (without
ootec) or males of red cockroaches, previously not starving
less than 2 days. Accounting for injured and dead insects and eating

baits by weighing them on analytical scales, determine
daily for 7-15 days. To identify an aversion (index
feeding) in variants with alternative feed
the value of the nutrition index (PI) by the formula 23.

Method of dosed feeding of insects.

The method provides for the feeding of insects from a micropipette with
drawn the end water solutions (emulsions),
prepared on 10% sugar syrup. The method allows to accurately
regulate the amount of insect-eaten solution by
interrupt feeding at the right time. With a constant volume
solution (for house flies - 0.01 ml, red cockroaches - 0.05 ml)
The dose of insecticide is varied by changing the concentration of DV. Before
feeding flies withstand without food 12 hours, cockroaches -
2 days. In control experiments, insects are fed with sugar syrup
or a solvent. The experiments are set in 3 replicates for each
concentration, in each replication not less than

10 insects. The process of dying off of house flies is observed in
for 1 day, cockroaches - for 3-5 days. Criteria for evaluation
the effectiveness of the insecticide is the value of $LD_{50(99)}$ expressed in μg
per insect or in μg per 1 g of insect mass (formulas 6, 7).

2.2.4. The method of studying the manifestation of the knockdown effect in insects under action of insecticides

A comparative evaluation of the manifestation of knockdown is carried out by express-method. The method is based on measuring the time from
treatment of insects before the onset of knockdown (CT_{100}, min -
the average for replicas for 100% of individuals, KT_{50} - average
on replicates for the first 50% of individuals). On non-traveled

cockroaches or 5-6 day adults of houseflies are applied to 1 μl
acetone solutions of insecticides. Then the insects are placed in
Clean glass containers with vertical walls (glasses or
test tubes).

Knockdown is considered fixed when the insect can not
stay on the vertical wall of the vessel. In the experience apply
3-5 concentrations of insecticides, differing in 2-10 times.
Repetition of the experiment 3-5 times, for each replication used
10 insects. Accounting for the time of the onset of knockdown
individually, planting insects on one individual in a test tube. When
work with cockroaches time of onset of the state of knockdown determine
separately in males and females. Indicator of the onset of the state
knockdown in insects is the inability to crawl up
glass surfaces, turning over on the back and breaking
coordination of movement. Note the time in minutes from the application
insecticide before the first signs of poisoning
(hyperactivity, impaired coordination of movements) and onset
the state of knockdown (deep paralysis). Take into account the dynamics
poisoning of treated insects (% of affected insects) in
for 24-96 hours depending on the species. To characterize
the state of knockdown (reversible, irreversible) is plotted.

The plotting is carried out in semilogarithmic coordinates, on the axis abscissa delay the time of approach knockdown (logarithmic scale), along the ordinate,% of affected insects. In case if The state of knockdown is reversible,% of affected insects decreases in time. If the knockdown state is irreversible - insects completely die.

2.2.5. Methods for studying the acaricidal activity of active substances

Assessment of the biological activity of acaricides may be was carried out on active untrained females of various species Ixodes ticks. It is preferable to use species for experiments, most prevalent and epidemiological value. For example, for Russian Federation the most epidemiologically significant species are the taiga (I. persulcatus) and forest (I. ricinus) ticks, which are the main carriers of tick-borne encephalitis and iksodovirus tick-borne borreliosis. It is desirable, but not required, Additional tests for male mites and species, which are secondary carriers. If necessary studies are conducted on several types of ticks, differing biology, geographic distribution and sensitivity to acaricides. It is preferable to carry out the tests in a period of high activity of ticks in nature and use of natural female population. Collect females from vegetation should be no more than day before the experiments and store them in test tubes differentiated humidity or in wet bandages when temperature 12 ± 2°C. It is possible to use laboratory females After checking their motor activity and aggressiveness.

The method for determining the rate of onset of a knockdown state and the height of the rise of the mites on the treated tissue.

This method is useful for assessing the activity acaricides, manifested in the first 15 minutes. impact. Such

data are needed to address the issue of the prospects of the substance

as an active substance in the means of individual protection of people from attacks of ixodid ticks.

In the experiments, a test was used from cotton coarse calico, which is a tape 10 × 70 cm, on which at a distance of 10 cm from the edge (further a mark 0) are put by a pencil of a mark of length downwards up to 60 cm. The tests are placed horizontally on non-absorbent surface (glass, ceramic tiles, etc.). On an experienced test from pipettes evenly onto the belt section, starting from 0 to 10 (the area of the treated area is 100 cm^2), 1 ml of 1% acetone solution of the substance under study. Benchmark test is treated analogously using acetone. After evaporation all tests are hung in the laboratory in the same controlled conditions of temperature, humidity, illumination.

The experiments are carried out on the day of treatment. The desktop should be is freed from excess items and is covered with white paper, that will reduce the likelihood of mite loss. The tests are fixed at an angle of 70 ° to the horizon. Ticks one by one are placed 5 cm below the zero mark and observe their movement up the fabric, additionally stimulating them with the finger of the observer who is holding at a distance of 0.5 cm from the hypostome. Time is recorded with stopwatch. On the control test of female taiga tick at The temperature of 22 ± 2 ° C must pass in two minutes not less than 25-30 cm. On the test test, the time from the moment crossing of the lower part of the treated area with the tick the mite falls off the dough, which corresponds to the onset time state of knockdown (CT, min.). It is often useful to perform Figures reflecting the movement of mites in the tests. Missing mites are placed in a 70% solution of ethyl alcohol for the purpose of

conservation or continue to monitor them further. An experience
spend at least 30 females. The mean value
time of occurrence of the state of knockdown CT Wed , minutes and
statistical error. Simultaneously with the CT cf.
Record the maximum height of the tick lift according to the test.
Calculate the average value of this indicator in centimeters of MB cp and
statistical error. It is expedient to compare the obtained
data on the acaricidal activity of substances with
performed earlier similar work.

Method for determining the rate of sucking ticks.

This method allows you to assess the change in aggressiveness
iksodovyh ticks in relation to the warm-blooded animal,
caused by the action of acaricide. It is known that many
Acaricides have the property of accelerating the sucking of ticks to
warm-blooded organisms.

On the carefully cut back of the laboratory rabbit
colloid or other non-toxic glue to the skin glue
next to 4 glass cylinders (diameter 3, height 4 cm). One cylinder
is control, and three - experienced. First in the control
The cylinder is started by 5 females that have crawled before for 2 min. on
untreated (control) test. Upper Cylinder Hole
tighten with a fine-mesh material, fixing it with rubber
ring. Time from start to suction of each female to a rabbit
recorded using a stopwatch.

In each of the experienced cylinders run 5 females at once
after their contact with the treated surface of the dough. Contact time
should equal ½ KT cp . Control and experience are repeated three times.
Calculate the mean value of the female sucking time in

control, experience and the ratio of these indicators, which is called
the suction speed index (ISI) by the formula 14. It is advisable
to compare the obtained data with those given in the earlier
works.

2.2.6. Methods for studying the repellent activity of substances by relation to bloodsucking insects and mites

The study of substances with repellent activity in arthropods, are carried out in two stages. At the first stage determine the level of repellent activity of 5, 10 and 20% solutions substances in ethyl alcohol in relation to standard laboratory cultures of mosquitoes Ae. aegypti and fleas of X. cheopis. Research in comparison with the standard, which is recommended a well-known repellent diethyltoluamide (DETA) in similar concentrations. At the second stage, the spectrum of repellent effects of selected substances at different concentrations with respect to Natural populations of various species of insects and ticks.

Since the toxicity of the substances being sampled, as a rule, has not been studied in full, for the experiments use special olfactometers.

The advantage of the recommended olfactometer is that several substances with unknown toxicity, standard and control. Repellent activity toxicologically studied repellent substances after appropriate conclusion on the safety of application to the skin human, it is advisable to study in the form of solutions in ethyl alcohol when applied to the skin of volunteers.

The method for determining the repellent activity of substances with respect to to the laboratory culture of mosquitoes in the olfactometer.

The method is based on the reaction of escape of mosquitoes during excitation and their striving to fly to the light. A distant repellent action of substances on mosquitoes.

On strips of filter paper or cloth (coarse calico) size 2 · 6 cm, apply 0.12 ml of 5-20% solutions of the test compounds or 0.5% solutions of synthetic and natural fragrances. After complete drying, the tests are placed in an olfactometer. Every the concentration is tested in triplicate. Control Strips

impregnated with 0.12 ml of solvent. For the experiments,
olfactometer, the main part of which consists of a cylindrical
camera height of 12 cm and a diameter of 30 cm. The camera has a circle
24 holes, into which tubes with a diameter of 2.5 cm and a length of 6-8 cm are soldered.
Pipes have side layers, and at the end T-shaped glass
receivers into which insects fly through the tubes. One end
The receivers are covered with milling gas, the other with a stopper. Inside
The lateral branches of the tubes are put in test strips impregnated
solution of the substance under study. Inside the camera there is a movable
cylindrical curtain 6 cm high, closely adjacent to the walls
olfactometer. When the shutter moves up and down simultaneously
close or open the side openings of receivers with
studied substances, allowing or preventing mosquitoes,
being in the chamber to fly into the tubes and react to vapors
repellents. The olfactometer is placed in a specially equipped
box, where they maintain the optimal conditions for mosquitoes:
air temperature of 26 ± 1ºC and relative humidity of 60-70%, and in
which has a constant light source - a lamp of 150 watts.

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500 hungry mosquitoes are placed in an olfactometer chamber,
lowering the shutter, side openings, include an electric motor and a lamp,
located above the camera. The camera rotates slowly around the axis
for lighting alignment. Exposition 15 min. During this time
most of the insects leave the chamber and are
tubes. To count the insects in the tubes, the curtain
close, and insects are euthanized. Degree of frightening
the action of the substance is determined by the number of mosquitoes that have flown into the tubes
with the processed and control strips, and calculate the CODE by
formula 12.

When determining the duration of a deterrent action
The treated strips are tested every 3-5 days until loss
of their repellent properties when the RCD becomes below 70%. When
the study of rapidly evaporating substances (fragrant substances, etc.)
experiments should be conducted daily.

Method for determining the repellent activity of substances in relation to the laboratory culture of fleas in an olfactometer.

The method is based on the ability of hungry fleas to jump and stay for a long time on wavering strips of paper or tissue. The contact repellent action of substances is studied.

On strips of filter paper or cloth (coarse calico) size 1.5 · 14.5 cm, 0.2 ml of 5-20% solutions of the test substances are applied or 0.5-1% solutions of synthetic and natural fragrances. After 24 hours, the tests are placed in a special olfactometer. Every the concentration is tested in triplicate. Control Strips impregnated with 0.2 ml of solvent.

Experiments are carried out in an olfactometer, which is a cylindrical chamber with internal cylinder. On the axis below the top

a rotating disc is located on the cover. On a disk in a circle There are 24 openings where glass tubes are inserted with unfolded upper edge, inner diameter of not less than 15 mm. The height of the outer cylinder is 15 cm, the diameter is 25 cm, 15 cm and 17 cm respectively. Tubes hang between the walls of the outer and inner cylinders.

During the experiment, the treated and control strips are placed in olfactometer tubes, alternating 1 control with 4-5 treated, and close the top with stoppers. At the bottom of the camera Between the outer and inner cylinders 500 hungry fleas. Exposition - 20 min. For an even distribution insects drive with tubes rotate with a motor at a speed of 10 revolutions per minute. Insects leave the camera and are distributed by strips in tubes. At the end of the exposition the motor is turned off, the tubes are quickly removed from the instrument, placed in test tubes located in a tripod, the fleas are euthanized and counted.

The degree of deterrent action of substances is determined by ratio of the number of fleas remaining on the treated and control strips. The repelling factor (RCD) is calculated by formula 12. When determining the duration of a frightening action The treated strips are tested every 3-5 days until loss

of their repellent properties when the RCD becomes below 70%. When the study of rapidly evaporating substances (fragrant substances, etc.) experiments should be conducted daily.

The method for determining the repellent activity of substances by relation to ixodic mites on the tests of tissue. The method is based on negative geotaxis (the desire to creep up), characteristic many types of ixodid ticks and, in particular, representatives of the genus

Ixodes. In the experiments, tests are used from cotton fabric (calico), which are 10 × 70 cm tapes, at which distance 10 cm from the edge (further mark 0) mark a pencil plot (areas) to be (to be treated) repellent. Tests are placed horizontally on a non-absorbent surface (glass, ceramic tiles, etc.). To the test test from the pipette evenly on the certain parts of the tape are injected with solutions of the substance under study in ethyl alcohol. The reference test is processed similarly to the experimental test, using a solution of DETA. The control test is processed solvent. After 15 minutes. after processing, the tests are hung vertically to dry in the laboratory in the same controlled conditions of temperature, humidity, illumination. Experiments begin after complete drying of the tests (after 1-2 hours after processing).

The tests prepared for testing are fixed at an angle of 70 ° to horizon. Ticks one by one are placed 5 cm below the zero mark and observe their movement up the fabric, in addition stimulating them with the finger of an observer who is kept at a distance 0.5 cm from the hypostome. Benchmark all mites should creep endless. When testing the test and benchmark tests register the number of mites crawling the treated area (or zone). After testing the mites are placed in a 70% solution of ethyl alcohol for the purpose of their conservation or continue further observation of them. Each experience is conducted from no less than 30 females. The results of the experiments are compared with the test results reference test. The COD calculated on the basis of the test data

on the day of treatment, characterizes the acute action of the repellent substance. To determine the duration of repellent action

(DRD), the test is repeated daily until the RCD remains equal to or above 90%.

There are two possible ways of processing the tests: by cutting concentration and the concentration gradient. In the first case A test is used on which at a distance of 10 cm from the edge (0 mark) mark with a pencil a strip 10 cm long (area 100 cm^2). On an experienced test from the pipette evenly to the marked area of the tape 1 ml of a 20% solution of the test substance in ethanol is applied. The benchmark is treated similarly to the test, using solution of DETA. Calculate the CODE, which in this case is equal to% mites that did not crawl the treated area from the number of mites in the experimental option. This is the simplest version of the experiments, convenient if necessary in a short period of time to experience a lot substances.

Gradient concentrations create a processing zone of 5 cm (an area of 50 cm^2) with one milliliter of 5-10-20-40% solutions repellent in ethanol. The first treated area begins from the mark 0. Processed zones alternate with control zones (untreated) strips of 10 cm.

The control test is processed using only the solvent. After evaporation of the solvent, all tests are hung in the laboratory at the same controlled conditions of temperature, humidity, illuminance. The experiments are carried out on the day of treatment. Tests fix at an angle of 70 ° to the horizon. Ticks one by one are placed 5 cm lower the first processed strip and observe their movement upwards On a fabric, in addition stimulating them with a finger of the observer which keep at a distance of 0.5 cm from the hypostome. On a control test, both Normally, all mites creep through the marked zone. During testing

Experimental and reference tests record the number of mites creeping each treated zone. Mark the behavior of ticks at intersection of the processed strips. Determine the smallest concentration, scaring away ticks. Satisfactory Repellent properties have a concentration at which The treated space is crossed by not more than 10% of the ticks, from the number taken in the experience.

After testing the mites are placed in a 70% solution of ethyl alcohol for the purpose of their conservation or continue further observation of them. Experience is conducted with at least 30 females.

Determination of the repellent activity of substances with respect to natural populations bloodsucking Diptera method olfactometry. The experiments use natural populations bloodsucking Diptera, dominant in this climatic zone. The design of the olfactometer is similar to that described above, but its the size and number of tubes depends on how many substances at the same time is also investigated with respect to which kinds of insects. When conducting experiments on mosquitoes and horseflies use an olfactometer, consisting of a 12-channel camera with a capacity of 1 liter and T-shaped tubes a diameter of 2.5-3 cm, a length of 8-10 cm. When carrying out experiments on the midges and an olfactometer with a 0.5 liter chamber and tubes with a diameter of 1.5 cm and a length of 8 cm.

The principle of the olfactometer is the same as in the laboratory experiments. Strips of filter paper or calico impregnate studied drug or solution of the substance. Because the scaring properties of substances and the reaction of insects to them vary under the influence of various factors, in every experience necessarily include a standard (DETA) and control. Each substance

are studied in 3 replicates. Control strips serve filter paper or calico, impregnated with a solvent.

Insects for experiments are collected in traps or by nets. To Insects less injured at gatherings, nets supply

receiver - a tube inserted into the narrow end of the net. AT
olfactometer is placed 500 midges or slugs, 100 mosquitoes or
50-100 flies. During the period of the experiments,
temperature, humidity, illumination.

The repelling factor (RCD) is determined by
ratio of the number of insects that have flown into the control tubes and
tubes with processed tests (formula 12). Compounds and
drugs that have a wide range of deterrent
actions with a RCD of at least 90% are selected for testing
on the treated tissue.

The method for determining the repellent activity of substances by
relation to natural populations of bloodsucking diptera
processing of tissue. Strips of gauze with a size of 20 × 50 cm
1000 cm $^{2 \text{ are}}$ impregnated with 10-20% alcohol solutions of repellents from
calculating 20 grams per 1 m $^{2 \text{ of}}$ tissue.

The first determination of the deterrent action of the tests is carried out
a day after treatment, the next - once every 3-5-7 days.
In the intervals between the tests, the tests are stored in a vertical
position in the open air under a canopy in conditions,
providing free evaporation of the substance.

Tests are conducted in places of mass attack of bloodsucking
of insects. Before the beginning of the research and during the period of the experiments
Insects are recorded and collected (net or
exhauster) to determine the dominant species, register

meteorological factors (temperature and humidity, strength
wind, light, pressure). Intensity of attack of bloodsuckers
per person is determined by counting the number of insects planted on
nude forearm (shin) of the tester for 20 min. (4 times
for 5 minutes) every hour during the period of daily activity
bloodsucking insects.

The effectiveness of repellent is determined in hours of maximum
activity of dominant species with the intensity of their attack on
nude forearm (shin) of the testator not less than 25-30 individuals

for 5 minutes. Testing is carried out by at least 3 people (one is testing
The prototype, the second - the standard, the third - the control). Testers with
Experimental and reference strips of tissue are located downwind
from the control at a distance of not less than 5 m from it and from
friend in conditions of uniform illumination. Strips of fabric are placed
on the exposed forearm (shin) and count the number of bloodsuckers,
sitting on them for 15 minutes. (three times for 5 minutes).

The amount of repellent action of the drug is determined by
indicator of RCD. An acute repellent effect is characterized by RCD,
established in the 1st day after the processing of the tests. As a reference
DETA is used in a similar concentration.

Determination of the duration of the repellent effect of the treated
the tissues are carried out periodically for 1-3 months. The substance is considered
Ineffective when its RCD goes below 70%.

2.3. Methods for determining the activity of insecticides.

2.3.1. Methods for assessing the effectiveness of insecticides,
designed to combat non-flying synanthropic
insects

A method for assessing the activity of a sticky trap without insecticides in
a kind of houses for fighting cockroaches. Evaluation of the effectiveness of sticky
Traps are conducted on the red cockroaches of the insect culture.

To do this, a box of Plexiglas 20 × 20 × 30 cm or
polygons 60 × 40 × 15 cm, on the top of the inner surface
which has a strip of Vaseline 2 cm wide, which prevents
crawling of insects. In the box (polygon), 20 females are placed,
20 males and 80 larvae of II-IV ages. Take off the paper and
release the sticky surface of the trap. Unseal the sachet
food bait and place it in the center of the trap (if the bait

is not trapped under protective paper). The trap is placed in
center of the box (polygon), in the box (polygon) have a drinker with

water and alternative shelter. Performance accounting is carried out through

1-5 hours, and then after 1, 2, 7 and 14 days and further if necessary.
The repetition of the experiment is threefold. At the same time,

option: in the box (polygon), place an alternative food on the substrate

(a piece of white bread with sunflower oil), a drinking bowl with water and.

alternative shelter.

Performance indicators: average catchability at day 7 -

not less than 90%; on the 14th day - not less than 95%; Trap resource - 1 redhead

cockroach / cm $_2$; validity period not less than 15 days.

Method for assessing the activity of insecticidal baits

used in the control of cockroaches (in the form of containers -

bait stations, tablets, balls, briquettes, granules, powder,

pastes, gels, etc.). Evaluation of the effectiveness of food baits

is performed on red cockroaches. For this, polygons are used

(container size of 60 × 40 × 15 cm), on the top of the inner

the surfaces of which are coated with a strip of vaseline 2 cm wide,

preventing the crawling of insects. The container is placed

on 20 females, 20 males and 80 larvae of II-IV ages of cockroaches; after

This is also provided with food bait or ready-made

application, or a weight of 0.5-3.0 grams on a substrate, a drinking bowl with water and

asylum. Simultaneously put a control variant: in the polygon

put food (a piece of white bread with sunflower oil),

drinking bowl and shelter. The repeatability of the experiments is threefold, at a temperature

20-22 ° C and relative humidity of 50-70%. Accounting for death leads

daily. To study the residual effect,

new experimental group of insects through 7, 14, 21, 28 and more

days.

Accounting for the bait's eating habits is led by its daily

weighing to within 0.001 g. Since the bait in the first

a few days, loses the moisture that enters the formulation, take into account

loss of mass of the facility in comparison with the mass of the control variant (in

absence of cockroaches).

Method for assessing the activity of insecticidal gels.

When testing gels, experiments can be laid in two

Modifications:

a) application of the gel in the form of a dotted line along the perimeter of the vessel (1.5 cm gel with 4-10 cm interval);

b) placing a 0.5-1.5 g gel on the substrate.

The tests are carried out in glass crystallizers (D = 30 cm). Red cockroaches are placed in a crystallizer of 20 pcs. (10 males and 10 females). Accounting is similar to the above.

Performance indicators:

a) DV - FOS, carbamates, pyrethroids, phenylpyrazoles (fipronil): acute action - death of insects on day 2 not less than 70%; duration of residual action - not less than 30 days;

b) DV - imidacloprid (gel): acute action - death on the 3rd day not less than 70%; the duration of the residual action is not less than 90 days;

c) DV - hydramethylnon or other hydrazone, boric acid, sulfuoramide, perfluorooctane sulfonate lithium, thiamethoxam, avermectins: acute action - death on day 5 at least 70%, the duration of the residual action is not less than 90 days;

d) DV - boric acid, borax (gel): acute action - death for 5 days not less than 70%, duration of residual action - not less than 90 days;

e) DV - boric acid, borax (liquid bait): acute action - death on day 5 at least 70%, duration of residual actions - not less than 10 days.

Method for assessing the activity of agents in aerosol or non-palletizing packaging in relation to non-flying arthropod. The experiments are carried out in chambers with a volume of 1 m^3, equipped with a ventilation system. The bottom of the chamber is lined filter paper. The temperature in the chamber experiments is 22-25 ° C with relative humidity not

less than 50% and not more than 70%. To measure the effectiveness of funds
aerosol and non-propellant aerosol packages with

a sharp action and period is determined by the mechanical sprayer
residual action.

Definition of acute action.

To determine the acute effects of imago red,
black and American cockroaches, which are placed in 0.5 liter
capacity of 10 copies (5 females and 5 males), bed bugs and
their 1-4-day embryonic eggs are placed in glass
Exposures (H = 5 cm, D = 4.5 cm), which are placed on the glasses
10 × 10 cm, coated with filter paper.

Insects and surfaces are irrigated with a drug from a bottle with
height of 20 cm, directing the aerosol jet at an angle of 45 ° to the bottom of the chamber.
The insects and the surface are removed from the chamber after 10 minutes. after
irrigation. Insects are transferred to clean dishes. For their condition
observe, marking at 5, 10, 30 minutes, 1, 2, 4 hours and further daily
for 2-5 days the number of insects without external signs of paralysis,
paralyzed and dead. Entomological evaluation of each
formulations of aerosol cans or non-propellant
packings are carried out in 3 replicates. Drug consumption rate
is 10-20 g per 1 m $^{2 \text{ of the}}$ treated surface, depending
from the formulation of the formulation. Deviation from the average value
the consumption of the drug should not exceed 5%. Drug consumption
are determined by weighing the container (package) before and after the test.

Determination of residual action.

To determine the residual effect of aerosol deposits in five
At the bottom of the chamber, test surfaces (glass or
plywood, or other types of materials measuring 10 × 20 cm. These
The treated surfaces after treatment and drying are stored in
vertical position at room temperature and periodically

they are imbued with imago red cockroaches and bed bugs
and others to determine the duration of the insecticidal action.
The residual effect of aerosol deposits is determined through
1, 2, 3 days and further in the presence of long-term residual action
every 7 days for 28 days or more after contact
cockroaches with treated surfaces for 15 min. from
using Nabokov-Laryukhina's exposure meters or for 1 hour and
more in exposures with D = 85 mm, according to the recommendations of CSMA.
Insects after contact with the treated plates are transferred to
clean vessels and record their condition for 24, 48 and 72 hours
depending on the chemical structure of the active substances.
Residual action is considered complete when mortality
insects is less than 50%.

Performance indicators: acute action - death of cockroaches
in 10 minutes. not less than 30%, after 24 hours - not less than 100%,
the duration of the residual action is 5-60 days.

Method for assessing the activity of pyrotechnics,
intended for combating flightless insects and adults
house flies.

Assessment of the effectiveness of pyrotechnics is carried out on
red cockroaches, rat fleas, bed bugs and room
flies of insect cultures in special experimental
rooms, as they are designed for indoor use
large volume, and it is impossible to violate the structure of the facility, since
This changes thermodynamics and results in distorted results
tests.

On the floor of the room at a different distance from the test substance
in 5 points are placed: containers with a volume of 200-500 ml, in which

20 adults of cockroaches (10 females and 10 males), glass
Exposure meters (H - 5cm, D - 4.5cm) with 10 bugs, high
glass vessels with fleas of 30 pcs., tents made of tulle with room

flies of 3-5 days of age for 100 pcs. During the tests
air temperature is maintained in the 22 ± 2 ° C mode, to avoid
exciting or depressing influences temperature,
relative humidity is maintained within 70-90%.

On substrate of non-flammable materials place
pyrotechnic means. The product is ignited, the room
covered, held for 2 hours, and then counted
affected insects and transfer them to clean dishes, accounting for death
carried out after 24 hours.

Performance indicators: acute action - death through 24
hours of cockroaches - at least 90%, fleas -100%.

Method for assessing the activity of insecticidal dusts for non-flying
insects and adults of houseflies.

Assessment of the effectiveness of insecticidal dusts is carried out on
Red cockroaches and other flightless insects and houseflies
insect culture.

Various weighed parts of dust to select the rate of application of this
means not based on 1 m^2, but on the area of the test surface are applied
on the plates of plywood size 10 × 20 cm (area 200 cm^2). Sling
The dust is evenly distributed over the area of the plate. Insects
are placed in the exposures on the treated surface for 30 seconds,
then they are transferred into clean glasses. Short term of the selected
contact is due to the fact that the dust quickly adheres to insects,
so do not increase the exposure, because the dust,
The insect on the body continues to act after

transplant them into a clean glass. In determining the acute effect
Insect infestation is carried out immediately after application of the dust. Accounting
deaths occur after 24-48 hours. When determining the residual action
insects are planted after 1, 3 or more days after treatment
surface until the end of the insecticidal action. Accounting for death
insects after a replanting also lead in 24-48 hours.

Performance indicators: acute action: death of cockroaches,
flies after 24 hours - 100%; duration of residual action -

7-90 days.

The method of assessing the activity of insecticide pencils, crayons, bars.

Used as a test surface of a plate of plywood size 10 × 20 cm (area 200 cm^2), which is weighed and the results are recorded. Preliminary calculate the rate of flow for the test substance not on 1 m^2, but on the area of the test surface, as mentioned above. Insecticide pencil or bar the test surface is painted over. To control the rate of consumption the treated plate is weighed. If necessary, a remedy remove or add. In determining the acute effect insects are placed in the extermometers immediately after application. funds for 30 seconds, then they are transferred into clean glasses. Accounting for death are conducted after 24 hours. In determining the residual effect of insects for 30 seconds. after 1, 3 or more days after treatment surface until the end of the insecticidal action. Accounting for death insects also lead in 24-48 hours.

Performance indicators: acute action - death of cockroaches in 24 hours 100%; the duration of the residual action is 7-60 days.

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Method for determining the activity of insecticide pencils, chalk, bar for cockroaches.

Take a container (pelvis or crystallizer) with a diameter of at least 30 cm. From the wrapping paper, cut out a circle whose diameter corresponds to the diameter of the pelvis. Preliminary marking with a pencil - 6 circles are applied. The diameter of the first is 4 cm, the diameter of each subsequent increases by 2 cm. The paper circle is weighed and the results write down. Previously count area 3 concentric bands on each circle according to the formula 15.

For our example, a simple calculation looks like this:

$$S = \frac{3.14}{2} (14^2 - 12^2 + 10^2 - 8^2 + 6^2 - 4^2) = 169.56 \text{ cm}^2$$

In accordance with recommended consumption rates

insecticide per 1 m 2 the surface to be treated
The amount of funds applied to concentric
band. Thus, at a rate of flow of 5 g / m 2 in the case under consideration
should be applied 85 mg funds. Further insecticidal
with a pencil or a shallow paint 3 concentric bands are painted.
To control the applied norm, a paper circle with
means again weighed. If necessary, the remedy is removed
or add.

When determining the acute action in the center of the paper circle,
placed in a container, the edges of which are lubricated with petroleum jelly, from a test tube
produce 10 imago red cockroaches (5 males and 5 females). Cockroaches
run up and hide under a paper circle. In this case, the contact time
insects with a treated surface is 3-5 sec. Then the circle

carefully take out and collect cockroaches in clean glasses. Accounting
They die in 24 hours. The experiments are set in 3-5 fold replicates.

In determining the residual effect, paper circles with
the deposited product is stored for 3 or more days in a horizontal
position away from sunlight. For determining
the duration of the residual action, the process described above
repeat after 3 or more days depending on the duration
residual action or selected by the experimenter exposures.

Performance indicators: acute action - death of cockroaches
in 24 hours not less than 50%; the duration of the residual action is 7-60
days.

Determination of the bullet activity of insecticides.

Described below ways of definitions bulbar
effectiveness of powdery and liquid insecticides may
not only in stationary laboratories, but also in field
conditions do not require the availability of sophisticated laboratory equipment and
can be used even in the field. Useful for
quick selection of the most effective insecticides
field and village pest control in any given region from

number of available drugs, as well as for the indicative
determine their residual effect.

Definition bulbar activity powdery insecticides. In four glass cylinders (two control cylinders) height of 25-30 cm pour clean sand layer of 3 cm. Then into the sand of two cylinders, the test drug is applied at the rate of the applied dose of DV for 1 m^2 of area (the area of the bottom of the cylinder is determined by the formula $S = \pi R^2$). The preparation is thoroughly mixed with sand. After that, 10 cylinders are placed in all the cylinders

(laboratory crops or natural populations), preferably type Pulex irritans. It is possible to use other types of fleas (Xenopsylla cheopis, Ctenocephalides felis).

The fleas are kept on the surface of the blown sand in depending on the group to which the tested insecticide: organophosphorus - 20 minutes, carbamates - 10 minutes, pyrethroids - 1 min. After this exposure on the substrate with dust Fleas are transferred to spare clean cylinders. Results of accounting Recorded through 3 (pyrethroids), 24 and 48 hours. Death of fleas in the control option should not exceed 5%. Experience is conducted in two replicates at a temperature of 20-26 ± 2 ° C and a relative humidity not less than 65%.

Performance indicators: the drug is considered suitable for field pest control if, after taking into account 3-24 hours, the death of insects is about 50%, and after 48 hours - 95-100%.

Determination of bullet activity by the method of pollinated test tubes.

Determination of biological activity of insecticidal dusts can be produced by a more simple method of pollinated test tubes. To do this, take 6 clean tubes, in 2 of them place on 10 control fleas. The remaining tubes fall asleep five-gram a piece of insecticide dust. Plugging holes in tubes, 3-4 times turn them so that the walls are pollinated evenly. After this dust is poured out and, turning the tubes upside down, light

tapping removes excess drug. Ultimately
the inner surface of the test tubes should look slightly
clouded with dust.

In the tubes prepared in this manner, 10 fleas are placed in each and sustain them with a reduced twofold, compared to with glass cylinders, exposure. After this flea are transferred to clean test tubes (every dozen - in a separate). The results of the experiment are recorded and evaluated in the same way as in the first case after 3, 24 and 48 hours.

Determination of the bullet activity of liquid insecticides.

For work you need an enamel basin, a paper circle, corresponding to the bottom of the pelvis, 6-8 tubes. In the experience are taken 60-100 fleas of the same species from the insectarium, or from natural habitats; 20 fleas were left in two test tubes as control, and the rest are used in the experiment.

The test drug is applied to the paper surface with using a brush or a sprayer in terms of recommended for premises concentration and dose of ADV on the area of the circle. After The paper circle is placed on the bottom of the pellet, processed surface to the outside. 30-50 fleas are placed on a paper circle and cover them with a Petri dish (diameter of Petri dish 10 cm) in avoidance of sprawl. After 30 minutes. contact with paper, treated with an insecticide, the fleas are shaken into the pelvis, from where place 10 specimens in tubes.

Tests are carried out in duplicate using the same impregnated paper circle. The result of experience counted after 24 hours. In this case, in two replicas, the death of fleas should not be less than 98%. In this case, the test the drug is considered suitable for disinfestation in the recommended dosages. The death of fleas in control should not exceed 5%. Experiences

at a temperature of 20-26 ± 2 ° C and relative humidity is not
below 65%.

If the percentage of flea death ranges from 50 to 98, then
it is advisable to double the concentration of ADV, or, if this is not possible,
doubling the amount of insecticide formulations on paper
circle. The obtaining of high efficiency in these cases gives
basis to apply this formulation for disinsection in
increased concentration. If and at an elevated concentration
puletsidnaya efficiency will be below 98%, then the insecticide should be
To recognize unsuitable for settlement pest control.

This method can also determine the minimum exposure
Insecticides, which provides the necessary indices of flea death. When
This is the time of contact with an impregnated paper circle
Fleas with this formulation can be accurately dosed
from the immediate (2-3 seconds) to the long-term.

Method for assessing the activity of funds used by methods
spraying (emulsion concentrates, microencapsulated, micro-
and macroemulsions, wettable and soluble powders, forms
flow, suspoemulsions, aqueous solutions, etc.) to combat
insects.

Evaluation of the effectiveness of concentrates from which workers are prepared
Insect control fluids are performed on cockroaches and other
insects of insect cultures. In the event that the remedy
is designed to destroy a certain type of arthropod,
tests are carried out at this facility.

A sample of the means presented to determine its
activity, it is intensively stirred up and on the scales a hinge is taken for
cooking a series of concentrations. By dilution with water

the necessary concentrations are prepared. Experiments are conducted on insects in
chamber with a volume of 1 m^3 , equipped with a ventilation system. At the bottom

The cells in the area of 0.5 m 2 are placed in a constant pattern evenly in 5 points of 10 specimens of cockroaches, (the ratio of females and males 1: 1) which are placed in 0.5 liter vessels, bed bugs and their eggs in the exposures described above.

To establish the residual effect of sediments, the means specimens of surfaces - plates of glass and plywood size 10 × 20 cm placed in the chambers in an amount of not less than 5 pieces each. Insect and surfaces are sprayed with a spray or sprayer type "Rosinka" or "Quasar" from a height of 20 cm, directing jet means at an angle of 45 ° to the bottom of the chamber. The temperature in the chamber is The time of the experiments should be constant 22 ± 2 ° C, the relative Humidity - not less than 50% and not more than 70%.

The insects and the surface are removed from the chamber after 10 minutes. after irrigation. The rate of flow of working fluid at the rate of 50 ml / m 2 . Insects are transferred to clean dishes. For their condition, noting at 10, 30 minutes, every hour and further daily for 3 days the number of insects without external signs of paralysis, paralyzed and dead. The consumption of the drug is determined or preliminary, taking 50 ml of working liquid during glass processing and 100 ml when processing plywood, or by weighing the sprayer before and after spraying.

Residual action of the deposits of the agent is determined by 1, 3, 5, 7 day and more after treatment by the contact method of cockroaches with treated surfaces for 15 minutes. (flies, fleas - 5 min.) in the exposition. Insects after contact with treated

The plates are transferred to clean vessels and their condition is recorded in 24-48 hours.

Performance indicators: acute action - death arthropods after 24 or 72 hours 100%, the duration of the residual actions - 7-60 days;

2.3.2. Methods for assessing the activity of funds used to combat ants

Assessment of the effectiveness of the means used to combat

ants spend on working specimens of red house ants

Monomorium pharaonis L. laboratory culture, in the absence

Insect culture - on individuals from natural populations, catching

working individuals are carried out in full-scale conditions with the help of

exhauster. It is possible to use ants of natural populations

other species - black garden Lasius niger L. and (or) ants

Myrmica rubra L. In the absence of ants, male red

cockroaches of sensitive laboratory races.

Means for destroying ants of contact type of action.

These drugs include all formulations

insecticides of the contact type of action designed for

destruction of a complex of flightless insects, an assessment of their activity

in respect of working individuals of ants are carried out by spraying

or free contact with the treated surface. Residual

The action is checked when the ants are planted weekly, until the

efficiency is below 70%.

Food baits for the destruction of ants.

Insecticidal food baits (liquid, in the form of bait

container stations, powder, granules, paste, gel, etc.) may be

nonspecific - destroying predominantly workers

individuals of ants and specific - destroying the entire colony.

The method for determining the insecticidal activity of baits for

working individuals of ants. Experiments are carried out using the group

feeding. To do this, depending on the type of bait used

Open Petri dishes, laboratory beakers, any other

glass or plastic containers of sufficient size. The edges

carefully lubricate with petroleum jelly. A bowl with water is placed in a container

(wet cotton wool), shelter (a sheet of paper in the form of loosely rolled

tubules), insecticide bait (powder, granules, paste, gel and

etc. on the substrate; container with bait; wetted liquid

cotton wool).

After placing the bait in a container run 100 workers

individuals of ants. To prevent crawling of ants, except
applying vaseline oil, the container is additionally covered
glass. Experiments are carried out under natural light-dark conditions and
room temperature in 3 replicates. In the control version
use a drinking bowl with water and feed (honey, jam, minced meat).

Observing the state of insects with paralyzed
(dead) individuals begin a few minutes after their application
in the container and continue for the first 1-3 hours, in order to
identify nonspecific insecticidal baits that
their very rapid action on workers or do not reach
colonies, or do not spread in it through trophallaxis.
Further, death records are taken every 24 hours for 2-5 days
depending on the type of active ingredient used.

When using male reds as a test object
cockroaches sensitive laboratory race duration

experiment can be increased to 21 days, the experiments are carried out in
polygons size 60 × 35 × 15 cm. If necessary, into the container
place alternative feed.

Performance indicators:

a) DV - FOS, carbamates, pyrethroids, phenylpyrazoles
(fipronil): acute action - death on day 2 at least 70%;
duration of residual action - not less than 30 days;

b) DV - imidacloprid (gel): acute action - death on the 3rd day
not less than 70%; the duration of the residual action is not less than 90 days;

c) DV - hydramethylnon or other hydrazone, boric acid,
sulfuoramide, perfluorooctane sulfonate lithium, thiamethoxam,
avermectins: acute action - death on day 5 at least 70%;
the duration of the residual action is not less than 90 days;

d) DV - boric acid, borax (gel): acute action - death on 5
a day not less than 70%; the duration of the residual action is not less than 90
days;

e) DV - boric acid, borax (liquid bait): acute
action - death at 5 days not less than 70%; duration of residual

actions - not less than 10 days.

The method of studying the activity of specific food baits on

colonies of red house ants.

To assess the effectiveness of the remedy for the entire colony

select only those funds that cause the death of at least 70%

workers, but not earlier than in 1-3 hours. Study

effectiveness of the means for the whole colony are conducted with

using colonies taken from the laboratory culture of redheads

house ants, which consist of several hundred (up to 1000)

Working individuals and several females with brood.

The colony is placed in a vessel of the aquarium type with a volume of 0.03-0.05 m3,

the edges of the walls are carefully lubricated with petroleum jelly. To the aquarium

place a drinker with cotton wool soaked in water, and insect feed

(boiled yolk, eggs, flies, boiled meat, fish), a sheet of paper in the form

loosely rolled tubules (a refuge for ants). Control

serve similar colonies placed in the aquarium. Liquid

Insecticide bait is poured into a 10 ml cup with cotton

tampon. Periodically, the tampon is moistened by pouring drinking water.

Other types of baits are placed either on a substrate of 0.5-1.5 g (can

several substrates in different parts of the experimental capacity), or

in the container. As the experiment continues, the bait follows

periodically replace, first of all, if a sharp

reduction in the number of approaches to it of working individuals, or in the case of

a noticeable reduction in the amount of bait. Accounting for insect death

(separately working individuals and separately females) and visual assessment

Reduction of the brood is carried out at certain intervals

time until the complete death of the colony (usually from several

weeks to 1.5-2 months).

2.3.3. Methods for determining the activity of insecticidal agents,
designed to combat flying synanthropic
insects

Method for assessing the effectiveness of sticky (sticky) traps in the form

sheets, ribbons and sticks to combat flying insects (flies,

mol, fire). Evaluation of the effectiveness of sticky sheets and sticks

It is carried out on house flies, clothes moths, fire insects

cultures. To do this, large cages 50 × 50 × 50 cm are used,

These are frameworks with gauze tied on them

cages or cages made of mill gas or a chamber with a volume of 1 m^3 .

Sticky sheets, previously disconnected or removed from them protective

a paper layer, placed one at a time in a cage or chamber. Sticky

A stick or sticky tape is released from the protective layer of paper or

Cases and also suspended in a cage or chamber. In the tank release

100 individuals, in the chamber - 300 individuals. The repetition of the experiment is threefold.

The number of adherents is counted in dynamics: 3-5

hours, 1 and 2 days and further, depending on the need.

Performance indicators: for indoor flies - medium

catchability for 2 days is not less than 95%, the life of the trap is 1 individual / cm2, the time

actions not less than 15 days; for fires and moths - medium

catchability for 2 days not less than 70%, traps resource 1 individual / cm2, time

not less than 30 days

Method for assessing the activity of food insecticide baits

(liquid, dry sugar, granulated) to combat flies.

Assessment of the effectiveness of food baits is carried out on

indoor flies of insect culture. For this purpose,

Large cages 50 × 50 × 50 cm, which are frameworks with

stretched on them gauze crochets or cages from the mill

gas or special boxes in the volume of 0.5-1.0 m^3 . In the center of the cage (box)

Place a container with a liquid bait or place it on a substrate

2-3 g of dry baits (powder, foam, paste, granules).

Sugar bait is applied to glass, plywood or other types

surfaces and allow them to dry, then put in a box or a cage.

Simultaneously put a control option: in the cages (boxes) are placed

a vessel with 5% sugar syrup and produce 100 flies. Consider death

flies after 1, 2, 4, 24 hours, and then, depending on the purpose of the experiment.

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The primary evaluation of baits can be done by express method

Determination of the effectiveness of bait in relation to houseflies:

The dry bait is placed on a piece of cardboard with an area of 1 cm^2 and put it on the bottom of the glass. In a glass one fly is launched, tightened Top gauze and fix the time of planting on the bait and time the onset of defeat and death of an individual. Repetition of experience 5-10 times.

Performance indicators:

a) DV - FOS (except chlorophos), pyrethroids, neonicotinoids: acute action-the flies' loss in 24 hours 100%; durability residual action - 15-60 days;

b) DV - chlorophos: acute action - death of at least 80% of flies in 24 hours; the duration of the residual action is not less than 14 days.

Method for assessing the insecticidal activity of agents in aerosol packing in relation to flying insects (flies, mosquitoes).

Evaluation of the effectiveness of aerosol containers is conducted on indoor flies or mosquitoes of an insect culture.

A chamber with a volume of 2 m^3 produces 300 insects, while test, the air temperature is maintained at 22 ± 2 ° C to avoid unnecessarily exciting or depressing influence temperature, relative humidity - not less than 50% and not more than 70%. At study of each compounding put not less than 5 experiments. Jet Aerosol from the balloon is sent to the chamber, the consumption of the mixture should not be exceed 1 g / m^3 . Using the stopwatch, the time (T) lesions of 99% of insects. The concentration (C) insecticide in air according to formula 16. The effect is determined by the pulse concentration (C × T), where T is the time of destruction of 99% of insects, for

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which set the arithmetic mean and its

confidence limits at P = 0.05.

The criterion for assessing the effectiveness of aerosols is conditionally accepted value of insecticide concentration in air C_{15}, mg / m^3, (formula 17), which causes the defeat of 99% of flies or mosquitoes in the conditionally accepted time is 15 minutes and Q_{15}, mg / m3 - the amount of mixture released from the balloon, causing damage 99% of insects in 15 min. (formula 18), as well as the value of KT_{50} -time lesions of 50% of individuals. The value of KT_{50} is calculated graphically (or by formula 22).

Performance indicators: acute action on flies C_{15} - no more than 15 mg / m3; Q_{15} - not more than 1000 mg / m3; CT_{50} - no more than 10 minutes.

Methods for determining the activity of funds in the form of spirals (et al. means fumigation type) to combat flying insects.

Evaluation of the activity of funds in the form of spirals for mosquitoes on the imago of yellow fever mosquito Ae. aegypti or basement mosquitoes Cx. pipiens molestus of insect cultures aged 14-20 days, deduced on carbohydrate nutrition; for flies - on 3-day adults of indoor flies M.domestica.

Method for determining CT_{50}.

In a clean chamber with a volume of 0.5 m^3, 100 ± 5 female mosquitoes The temperature in the chamber is 25 ± 2 ° C. The spiral is ignited and after 30 seconds. The flame is blown out, the smoking spiral is placed in the camera and immediately turn on the stopwatch. Using the stopwatch register the time of the start of knockdown - paralysis of the first mosquito (1%) in chamber and the knock-down time of the penultimate mosquito (99%). For these indicators are determined graphically by time

knockdown in 50% of individuals (CT_{50}, min.). The experiment is repeated three times. Calculate the average value of CT_{50} graphically (or by formula 22).

Method for determining CT_{50} for indoor flies.

Evaluation is carried out in boxes or special rooms with a volume of not less than 10 m^3. Flies of 100 individuals in cages, of fine meshed mesh (tatin) are placed at a height of 1.5 m at three points in the room. An experience is carried out at a temperature of 25 ± 2 ° C. The spiral is ignited and after 30 seconds.

sustainable combustion, the flame is blown, immediately turn on
stopwatch. Using the stopwatch, record the start time
knockdown - paralysis of the first fly (1%) and knockdown time of the penultimate
flies (99%). According to these indicators, the graphic method determines
time of approach of knockdown in 50% of individuals (CT_{50}, min.). An experience
repeat three times. Calculate the mean CT_{50} value graphically
(or by the formula 22).

Method for determining mortality for houseflies.

After an exposure for 3 hours, the cages are taken out in a clean
room and left for 24 hours to account for the mortality of insects.

Method for estimating the spiral reserve.

To determine the spiral reserve (maximum time
use of a spiral) burn the helix completely on the open
air, determine the time of complete combustion.

Indicators of the effectiveness of spirals:

a) CT_{50} for mosquitoes - no more than 7 minutes; spiral reserve for
mosquitoes - not less than specified in the standard documentation;

b) CT_{50} for flies - not more than 60 minutes; mortality after 24 hours - not
less than 80%; reserve means for flies - not less than specified in the standard documentation.

Fumigation agents for fighting mosquitoes in the form of
insecticide papers, lantern plates with a candle, impregnated
volatile insecticide of polymeric carriers, etc.

Similar facilities tested at indoors
recommended by the manufacturer of the volume. Determine the CT_{50}.
(graphically or according to formula 22) and insect mortality after 24 hours.

Performance indicators: CT_{50} for mosquitoes - no more than 30 minutes;
mortality after 2 hours is not less than 80%. Reserve mosquito - not
less than specified in the normative document; when tested in a KT_{50} chamber - no more than 2 minutes.

Method for assessing the insecticidal activity of anti-mosquito preparations
in the form of candles.

Experiments are carried out in the laboratory at

temperature 20-25 ° C in experimental plastic chambers
volume of 1 m ^{3} , the surfaces of which must be without joints
mosquitoes could not hide in them. The object of research are
Imago mosquitoes Ae. aegypti and C. pipiens of insect cultures.

100 imago mosquitoes are admitted into the chamber, in the center of the lower
On the surface of the camera, place an experimental candle, which is ignited.
The candle should burn no more than 6 minutes, after which it is extinguished. When
the experiment, the temperature of the air in the chamber is fixed to
the beginning of the experiment and after the candle was doused. Before
ignition and after suppression the candle is weighed. Repeatability
The experiment is threefold. As a control option
Use a standard candle without an insecticide. Dynamics of death
mosquitoes are monitored at intervals of 5, 10 and 15 minutes. Then calculate
the values of KT $_{50}$ and KT $_{90}$, min. (the burning time of the candle providing
death of 50% and 90% of mosquitoes within 15 min. after it is extinguished), and
value of CT $_{50}$, min. (by the formula 22 or graphically).

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Performance indicators: death of mosquitoes after 15 minutes. after
candle suppression is not less than 90%.

Methods for determining the activity of electrofumigators for fighting
with flying insects.

Assessment of insecticide activity for mosquitoes is performed on adults
yellow fever mosquito Ae. aegypti or basement mosquitoes Cx.
pipiens molestus of insect cultures at the age of 14-20 days,
derived from carbohydrate nutrition; for flies - for 3-day adults
houseflies M.domestica.

Electrofumigators with mats (plates).

Method for determining CT $_{50}$.

In a clean chamber with a volume of 1 m ^{3} (for flies - 2 m ^{3}) run
100 ± 5 mosquitoes (200 ± 10 imago flies) without division by sex. Temperature
in the chamber is 25 ± 2 ° C. Electrofumigator (heating device)
are included in the electrical network according to the instructions in 15 minutes. before
the beginning of the experience. Insecticidal mat (plate) is placed on
heating surface and immediately place the electric fumigator with

plate in the chamber with mosquitoes. Using a stopwatch, register
the time of the start of the knockdown of the first mosquito (flies) in the cell and the time
knockdown in 99% of mosquitoes (flies). For these indicators, graphic
by a method or by the formula 22, determine the time of onset of knockdown
50% of individuals (CT_{50}, min.). The experiment is repeated three times. Count
the average value of CT_{50} (by formula 22 or graphically) and
statistical error.

Method for estimating reserve mat.

To determine the reserve mat (maximum time
use mat) determine the CT_{50} for mosquitoes and flies (for
(see the above method) using mats that have already been heated

before the experiment 1, 2, 3, 4, 5 or more hours. The experiment is repeated three times.
Calculate the mean CT_{50} (graphically or according to formula 22) and
statistical error. For reserve mats the maximum
heating time of the mat in hours, at which the CT_{50} is no more than
7 min. for mosquitoes and 9-20 min. for flies depending on the studied
active ingredient.

Liquid electrofumigators.

Method for determining CT_{50}.

The electric fumigator is included in the electrical network according to
instructions and after 60 minutes. place it in a cell with mosquitoes
(flies) in the same way as described above for electrofumigators with mats.
Determine the CT_{50}, min. as described above. The experiment is repeated three times.
Calculate the mean CT_{50} (graphically or according to formula 22) and
statistical error.

Method for estimating the reserve of fluid in the vial.

To determine the reserve of fluid in the vial (maximum
time of use of the liquid in the vial) determine the amount
evaporated liquid after continuous operation of the electric fumigator
for at least 8 hours. This process should not be repeated
less than 3-5 times. Each time the bottle is weighed before and after use,
fixed time of operation, Δ mass of the vial. Based on these
data calculate the amount of content released into the air
vial, mg / hour, and then reserve the vial in hours by dividing the mass

net vial in mg by the amount released into the air in mg / h.

Electrofumigators with a tablet.

As a DV carrier, a tablet of technical
plastic frame, which is used with electric heating
device of a certain type. The plastic frame is mounted

indicator (with a colored liquid). When the liquid in the indicator
completely evaporates, it is a signal to the fact that all the DVs are released into the air, and
the means has completed its work.

Method for determining CT_{50} .

The heating device is connected to an electrical network
according to the instructions in 15 minutes. before the experiment without a pill. Tablet
is inserted into the heating device and immediately placed in the chamber with
mosquitoes. Determine the CT_{50} , min. as described above. Experience repeats
three times. Calculate the mean CT_{50} (graphically or
formula 22) and a statistical error.

Method for estimating the reserve of a tablet.

To determine the tablet reserve (maximum time
use of a tablet) determine a CT_{50} for mosquitoes and flies (according to
the above method) using tablets that are already
heated up to experience for a certain time. An experience
repeat three times. Calculate the mean CT_{50} (graphically
or by the formula 22) and a statistical error.

Performance indicators:

a) with mats (plates): KT_{50} for mosquitoes no more than 7 minutes;
reserve mosquito mat for at least 7 hours;

b) liquid: CT_{50} for mosquitoes no more than 4-5 minutes; reserve
means for mosquitoes not less than specified in the standard documentation; KT_{50} for flies no more than
9-12 min. (when using vaportrin) and 20 min. (with
use of other volatile pyrethroids); means for flies
not less than specified in the normative document;

c) with a tablet: CT_{50} for mosquitoes no more than 15 minutes; CT_{50} for flies is not
more than 45 min. (when using transfluthrin); reserve funds for
mosquitoes (flies) not less than specified in the standard specification

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Method for evaluating the possibility of using emulsion concentrates and in aerosol or non-propellant packaging for processing vegetation (places of mosquito nets in nature).

To study the effectiveness of using blood-fed females (1-2 days after feeding on mice) mosquitoes Ae. aegypti (or etc.) of laboratory cultivation.

In a low laboratory dish (Petri dish) sprouted wheat or other cereals before shoots reach a length of 15 cm, after which plants are sprayed with the studied agent, dried in for 1 hour and put a "lawn" in a chamber of 0.5 m^3 from a mosquito imago. AT the control chamber is placed with untreated grass. Accounting affected insects spend an interval of 5-15 minutes. to the full death of insects.

Performance indicators: 100% death of mosquitoes when recorded through 1 hour.

2.3.4. Methods for assessing the activity of the means for combating preimaginal stages of insects

Method for assessing the activity of the means for combating mosquito larvae.

Assessment of insecticide activity for mosquito larvae is carried out on the larvae of the II-IV age of the yellow feverish mosquito Ae. aegypti or the basement mosquito Cx. pipiens molestus of insect cultures.

In vessels with a volume of 200-500 ml pour 100-400 ml tap water, stand-by for 24 hours. In each vessel (2 hours prior to the experiment) 20 larvae of II, III or IV age. After 2 hours of lost or weakened larvae, remove and replace by viable. Add 1 ml of solution to the vessels (emulsion, suspension) of an insecticide of a certain concentration.

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Simultaneously, 4-5 concentrations are tested, each in 3 replicates.

Control is provided by larvae in the water without addition
insecticide. In parallel, they put the control with the reference preparation. AT
period of the experiment, the water temperature should be within
21-23 ° C. Calculation of the dead larvae is carried out after 24 hours. If more than
10% of the larvae in the control pupated, the experiment was not taken into account and repeated.

Performance indicators: residual action - death of larvae
when administered on day 3 after 24 or 72 hours, 100%;

a) microbiological SC_{50}, mg / l - no more than given in TU;

b) on the basis of urotropine, etc. - loss of larvae after 24 hours
100%;

c) FOS, pyrethroids, etc. - loss of larvae after 24 hours 100%;
the duration of the residual action is 3-15 days.

The method of insecticide treatment of substrate with larvae
house flies.

In a 0.5 liter vessel with 200 g of wet bran is placed on a
substrate surface 30 larvae of III age. When all the larvae
penetrate into the substrate (after 10-15 minutes), from the spray gun evenly
water the surface of 10 ml of the solution (emulsion, suspension)
insecticide, which corresponds to 2 l / m^2 of the working fluid. Vessels
cover with calico napkins, which are fixed with elastic bands.

In each experiment, 5 concentrations of insecticide
in 3 replicates. The results are counted after 48 hours,
counting the number of larvae perished in 3 vessels, and in 3 vessels
The larvae are left in the substrate until the end of metamorphosis.
The efficiency of the treatment is determined by the percentage ratio
the number of dead larvae and the adults in control and experience.

Determine the concentration providing 50, 95 and 99% mortality
larvae (formula 3).

The method of substrate treatment with insecticides before larvae replanting.

Wet bran is treated with a solution (emulsion,
suspension) of the insecticide at a rate of 50 ml per 1 kg of substrate and
mix thoroughly. In every 200 g of the treated substrate

30 larvae of III age are planted. In each experiment,
4 concentrations of insecticide in 3 replicates. Profitability Analysis
spend 24 and 48 hours after the experiment. Remaining in
live larvae transplanted after 2 days in unprocessed bran and
observe the cycle of metamorphosis - count the number of pupae and
of the adults. The efficiency of processing is determined by
ratio of the number of larvae and the adults in the control and
experience (formula 3). When calculating the results of the experiments,
dose of DV for 1 kg of substrate.

Method of studying the effects of insecticides on pupae of flies.

For 10-20 pupae of houseflies are placed on the bottom of glass
vessels and covered with a layer of sand 6-7 cm high, then using
The spray is treated with sand with aqueous emulsions (solutions,
suspensions) of the preparation at the rate of 1 liter of solution per 1 m^2. Processing
spend 5-7 concentrations of the drug, each experience pose
in 3 replicates. Vessels with dolls are tied with gauze
napkins or put open in gauze (mesh cage).
Counting of the produced flies is carried out 2-3-5 days after
processing. At the same time put the control - vessels with dolls,
treated with a solvent (water). Effectiveness of the drug
are determined by the percentage ratio of the number of flies

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control and experiment (formula 3). Determine the concentrations,
providing 50, 95 or 99% of the death of flies.

2.3.5. Methods for determining the ovicide properties of insecticides

A method for studying the effects of insecticides on fly eggs.

When determining the ovicide of substances or agents for eggs, flies
you can use different methods. With the first method of laying eggs, flies
20 mg are wrapped in a cambric tissue and immersed in a solution
insecticide, then thoroughly rinsed in running water. Part
is left on wet filter paper for observation, and part
is transferred to a nutrient substrate. In the control variant of the egg

immersed in water. Accounting is done after 48 hours.

In the second method, 20 mg of eggs of houseflies are placed on wet bran in 0.5-liter vessels. For cooking wet Bran is taken on 100 g of calcined 200 g of water and carefully is stirred. In each vessel, 200 grams of wet bran are placed, the surface of the substrate with oviposition is uniformly irrigated with 10 ml solution (emulsion, suspension) of the insecticide, which corresponds to the norm flow rate of working fluid 1 l / m^2 . The dose of the drug is calculated according to DV per 1 kg of substrate. Experiments are put simultaneously at not less than 5 doses, each in 3 replicates. In the control variant oviposition is irrigated water or solvent. After irrigation, egg laying falls asleep A thin layer (1.0-1.5 cm) of wet bran to prevent them drying. Vessels are tied with calico napkins. Profitability Analysis spend 48 hours, counting the number of larvae that have proliferated from control and processed oviposition. Effectiveness of the drug are determined from the ratio of the number of larvae in the treated and

control substrate. Determine the dose providing 50% and 99% the death of eggs.

At the third method on plates of black paper 1 × 1 cm, slightly moistened with milk or a protein of chicken eggs, a brush or The dissection needle is applied to 20 viable eggs. Plates with the eggs are immersed for 3 seconds. in alcohol or aqueous working solutions means or active substances; control - in pure alcohol or water, dried on filter paper and transferred to cups Conveyors, in which the circular tanks are filled with water to maintain 100% humidity. To find the values of SC_{50} and SC_{95} , not less than 5-6 concentrations in 5-7-fold repeatability.

Method of studying the effects of insecticides on egg bugs.

Oviposition 3-5 days old, placed on sheets a filter paper of 30 cm^2 size is irrigated with 0.3 ml of a solution (emulsion, suspension) of the insecticide. Each insecticide is tested not less than 5 concentrations, in triplicate each. Dose

insecticide according to DV is calculated in grams per 1 m 2 of treated surface. In control, oviposition is treated with water or

solvent. After processing, eggs are transferred to glass

glasses and top covered with cut filter paper. Accounting

the results are carried out after 5-7 days. Effectiveness of the drug

are determined from the ratio of the number of larvae hatched from

processed and control oviposition. Determine the dose,

providing 50, 95 and 99% mortality of eggs.

A method for studying the effects of insecticides on flea eggs.

At the bottom of a three-liter jar, sheets of black paper are placed, so

so that it completely covers the bottom of the vessel and is placed there white

mouse in a cage. On the animal 100 females and 25 males

fleas. The sheets of paper are changed daily and the quantity

laid eggs on them. From the leaf on which eggs of fleas are laid,

cut out sheets of 4 × 5 cm and treat each with 0.2 ml

solution (emulsion) of the insecticide. Experiments are made in 5 concentrations, in

three replicates each. In the control version, a sheet of paper

treated with a solvent. Experiments with oviposition of insects

spend at room temperature - 23-27 ° C. Accounting

the results are carried out after 4-6 days. Efficiency of processing

are determined by the ratio of the number of larvae

processed and control sheets. Determine the concentration,

providing 50, 95 and 99% mortality of eggs.

2.3.6. Methods for assessing the activity of pediculicidal drugs

In the experiments, adults and larvae of the III age of lice

(Pediculus humanus humanus L., 1758) laboratory sensitive

culture or from natural populations harvested from clothing

infected lice of people who turned to the hospital. When

the need for experiments on head lice Pediculus

humanus capitis DeGeer use insects harvested from humans.

In the case of using a laboratory sensitive culture in

Experiments use well-fed lice. Experimental lice are kept in

thermostat at a temperature of 28 ± 2 ° C and relative humidity

70-76%.

In the case of lice from natural populations, experiments must be delivered on the day of collection of insects. Lice are harvested in glass entomological test tubes (not less than 50 mm high, diameter of less than 10 mm) for 20-30 individuals per each. Lice collected from one person, are considered as one micropopulation and

test tubes with them are labeled with one serial number. During delivery of the tube is taken vertically to prevent spillage lice. During the cold season insects in the laboratory need deliver in a refrigerator bag with inwards containers with Hot water to prevent hypothermia of lice.

For the standardization of biological material, each microdopulation of lice should be characterized in terms of their sensitivity to pyrethroids (permetrin) and FOS (malathion) in diagnostic concentrations. With a small amount assembled insects it is permissible to use a mixture in experiments lice from different micropopulations, with mandatory identification sensitivity of insects (from mixed micropopulations) to permethrin and malathion.

Method for determining the proportion of insecticide-resistant individuals lice.

For the experiments, standard filters with a diameter of 11 cm or cut a circle with a diameter of 11 cm from the filter paper. On filter, pre-writing on it with a pencil title insecticide, pipette is evenly applied to the acetone solution insecticide at a rate of 1 ml / dm_2 (0.95 ml per standard filter diameter 11 cm) in the diagnostic concentration (permethrin - 0.206%, malation - 2.02% in terms of 100% active substance), then it is dried in a suspended state until it evaporates completely acetone. After this, the filter is placed inside the lid from the cup Petri (diameter 10 cm), so that it snug against the bottom, and poured out on his lice. To insects do not crawl, they cover the remaining half a Petri dish, writing down the serial number of the test tube

with lice and the time of the beginning of the experiment. After 6 hours a cup

turn over and lightly tap it on the table. Lice that do not
can be held on the surface of paper, considered paralyzed
(in a state of knockdown). The share of active individuals remaining on paper
total number of insects placed in a Petri dish initially,
reflects the proportion of lice resistant to insecticide that were
the filter paper is processed. Experiments by definition
stability of lice to permethrin and malathion are carried out in parallel with
study of pediculicidal activity of agents.

When studying the effectiveness of insecticides, methods
Immersing insects and eggs or contacting them with processed
surfaces. The experiment is conducted at least three times, in each experiment not
less than three replicates. Insects and eggs are the control,
exposed to the solvent, and insects and eggs, not
exposed to an agent and a solvent (biocontrol).
After contact with pediculitis, the test lice are kept in plates
Petri on clean samples of calico 5 × 5 cm or in penicillin bottles
with pieces of calico 1 × 1 cm in a thermostat at a temperature of 28 ± 2 ° C and
relative air humidity of 70-76%. Accounting of the deceased in the experience
lice are carried out after 24 hours, head lice in 6 hours.
Accounting for the death of eggs (ovozidnoe action) is carried out weekly in
for 21 days. If the control killed more than 5 to 20%
insects or lice eggs, the experimental data are recalculated according to formula
Abbota (formula 4). At death in control of more than 20% of individuals, the experience is not
account and repeat.

The immersion method for adults, larvae and lice eggs for evaluation
insecticidal, larvicidal and ovicidal activities
pediculicidal means.

Prepare working fluids (aqueous emulsions, suspensions,
soaps, etc.) or use ready-to-use forms
(shampoos, lotions, etc.). Insects (larvae of III age, as
most resistant to insecticides, imago) of 20 individuals and eggs,
is placed in 5 x 5 cm calico wipes and immersed in working fluids
funds for different periods (from 10 minutes or more) to determine
necessary exposure.

In assessing the means to combat
pediculosis, strands of hair with head lice on them
(or polyamide fibers with clothing lice) are immersed in working
solutions or ready-to-use liquid preparations.
The duration of contact is from 1 to 60 minutes. (and more).

At the end of the exposition, the insects are washed with warm water
soap or shampoo for washing hair and re-warm water,
dried on filter paper and transferred to Petri dishes on a
pure samples of coarse calico 5 × 5 cm and placed in a thermostat at a temperature of
28 ± 2 ° C.

The method of contacting lice with treated surfaces
for the study of insecticidal and larvicidal activities
pediculicidal means.

In assessing the means designed to combat the clothing
pediculosis, samples of calico size 10 × 10 cm are treated with
(emulsion, solution, suspension, dust). Liquid forms
is used at a rate of 100 ml / m^2 , the dust is uniformly distributed over
The surface of the fabric is at a rate of 10 g / m^2 . Samples of coarse calico,

treated with a liquid agent, after drying, place in cups
Petri and on them are planted not less than 20 individuals of clothing lice (imago
and third instar larvae). The time of contact of insects with the agent
is from 5 to 60 minutes. depending on the purpose of the experiment.
Then the lice are transferred to Petri dishes for pure 5 × 5 cm calico samples

or in penicillin bottles with pieces of calico 1 × 1 cm and placed in thermostat at a temperature of 28 ± 2 ° C.

When establishing the effectiveness of pediculicidal ointments or Gel them with a brush applied to the surface of a tissue size 10 × 10 cm insects are planted for a period of 1 to 60 minutes. For determine the ovicidal activity of the drug is applied to pieces of tissue with eggs louse lays. The drug consumption rate is 1-5 g / 100 cm^2 . After exposure to a vaseline-based ointment or insect gel or their eggs are washed with warm water with the addition of soap or shampoo for washing hair.

Method for assessing the activity of pediculicidal agents in aerosol or non-propellant packages.

Fabrics with insects planted on them (imago and larvae III age), as well as oviposition, irrigate with an aerosol spray, directing it from a height of 10-20 cm at an angle of 45 ° C. Consumption rate means 20 g / m^2 . Amount of application is determined from the difference in weight of the balloon before and after the mixture is discharged and further calculate the consumption of the active substance, based on the composition the contents of the package. After irrigation of the tissues after 10 minutes. Experimental insects are transplanted into clean Petri dishes on clean pieces of coarse calico size 5 × 5 cm and placed in a thermostat for observation. Accounting for the death of clothing lice lead after 5, 10, 15, 30 minutes. and 1-5 and 24 hours, head - up to 6 hours.

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Method for assessing the effectiveness of impregnated tissues for the purpose of preventive maintenance of a pediculosis.

Impregnation of tissue samples.

To assess the insecticidal activity means, intended for impregnation of tissue for the purpose of prevention pediculosis, prepare samples of tissues measuring 10 × 10 cm. Samples are cut from moisture-absorbing tissue - cotton (cotton) knitwear and not moisture-absorbing - cotton. The number of samples of each The type of tissue is determined by the number of insecticide concentrations in experiment (at least 2-3 concentrations in three replicates on two

types of tissues). When studying a new active substance (DV) or
mixtures of DW in experiments use not less than 3 concentrations of insecticide.

Samples of tissue are immersed in freshly prepared liquids in working
concentrations (aqueous emulsions, suspensions, solutions). Liquid with
samples are thoroughly mixed with hands in rubber gloves,
periodically compressing the fabric for better impregnation. Working volume
solution of insecticide depends on the number of samples and the type of tissue and
averages 300-500 ml. After complete soaking
samples (5 min.) they are removed from the working fluids, squeezed and
dried.

After complete drying of the tissue, part of the processed samples
3 samples for each concentration of the insecticide) are used for
determination of acute and residual (when stored) insecticidal
actions. When determining the storage period for impregnated laundry
Samples are stored in polyethylene or kraft bags at
room temperature.

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The method of studying the rate of damage to lice in contact with
impregnated cloth.

On the dried samples of tissue,
Nabokov and plant at least 10 lice (imago and larvae
III age) in each. Signs of poisoning of insects register
every 15-30 minutes. With the defeat (immobilization) of all individuals in
experiment, contact is stopped and the lice are transferred to Petri dishes on
clean pieces of coarse calico size 5 × 5 cm or in penicillin bottles with
1x1 cm. These cups are placed in desiccators with
a saturated solution of table salt (relative humidity
air about 75%) and remove them into a thermostat (temperature 28 ± 2 ° C).
The account of the death of the louse is carried out after 24 hours. All experiments
is carried out in triplicate. All experiments accompany
control variants, in which lice are planted on
untreated tissue samples.

If in contact with treated tissues for 180 min.

remain active (able to move) individuals, then the means in this concentration is considered ineffective and the experiment terminate.

If contact occurs within 180 min. with treated tissues all insects lost motor activity, but when recorded through 24 hours left alive lice (even one individual), a remedy in this concentrations are considered ineffective.

Method for determining the acute insecticidal action of tissue, impregnated with pediculicide.

Nabokov exposures put lice on 20-30 individuals in each. Exposure is 60 minutes. Then the lice are transferred to Petri dishes on clean pieces of coarse calico size 5 × 5 cm or

penicillin bottles with a nested cloth 1x1 cm. These cups are placed in desiccators with a saturated solution of common salt (relative air humidity of about 75%) and remove them in Thermostat (temperature 28 ± 2 ° C). Accounting for the death of clothing lice conduct in 24 hours. All experiments are carried out in triplicate. All experiments are accompanied by control options in which lice are placed on untreated tissue samples.

If after contact 60 min. with treated tissues when recorded In 24 hours there were live lice (even one individual), a remedy in this concentrations are considered ineffective.

To establish the duration of residual pediculicidal effects of impregnated tissue during storage insecticidal properties are carried out every 7-10 days for a month. For of establishing residual pediculicide actions impregnated clothing when wearing samples of coarse calico and knitwear, treated with an insecticide at a selected concentration, The amount and quantity of volunteers duration of the experiment. After 3, 5 and / or 7 days with clothes 3 pairs of coarse calico and knitwear are spun in the selected insecticide concentration and control. They are planted with insects and evaluate the dynamics of poisoning and acute action. After studying

the effectiveness of the sample (lice transplantation) is withdrawn from the experiment, not sewing again to clothes. The last experience is carried out in time, corresponding to the maximum period of continuous carrying clothing made of impregnated fabric, toxicologists for each particular remedy.

Performance indicators:

a) funds for the treatment of head lice (lotions, shampoos, soaps and other detergents, dusts, creams, gels, concentrates emulsions) and lapidary lice (emulsion concentrates): death imago of lice in 24 hours 100%, death of eggs after 21 days 10-100%;

b) means for impregnating laundry to prevent pediculosis: acute action - death of adults and larvae III age of lice in 60 minutes. exposition on impregnated tissue (accounting after 24 hours) - 100%; cessation time activity in 100% of individuals during contact with an impregnated insecticide cloth - no more than 180 minutes; duration of residual actions when wearing - at least 3-7 days.

2.3.7. Methods for assessing the activity of means of struggle with keratophagous insects

Evaluation of effectiveness is carried out on laboratory cultures imago and caterpillar tracks of T. bisselliella moth and imago and larvae of A. A. smirnovi. Means for the control of insects-keratophages are subdivided into nonspecific and specific.

To nonspecific means for combating butterflies moths means in aerosol and non-propellant aerosol packaging. For experiments use 1-2-day butterflies moths. Experiences are carried out in glass chambers with a volume of 2 m_3 , or are used modification of the method, according to which the tests are carried out in wardrobes, with a volume of approximately 1 m_3 , into which 50 butterflies moths and spray the product at a rate of 1 g / m_3 . Accounting for death

is carried out after 15 minutes. and after 24 hours, given the number of live, dead and paralyzed butterflies.

To nonspecific means of combating larvae of tadpoles means of a contact type of action designed for destruction of complex flightless of insects. Evaluation of The effectiveness of these means is carried out with respect to larvae kozheeda 11-12-week age of method 15-minute forced contact with the treated product surface.

Method of studying insecticidal and ovicidal activity specific means of contact type of action.

Acute action is determined by direct irrigation means of 28-30 day moth caterpillars or 11-12 week old larvae of the kozheed on the food substrate - unapproved cloth (article 3907), as well as the method of contacting insects with the surface 10 × 10 cm, treated with the agent, based on from the rate of consumption of 10-20 g / cm^2 (until lightly moistened). As control uses similar samples, processed only the main solvent present in the medium. Treated the samples are dried for 24 hours at room temperature, put on them 10 caterpillars of moths or larvae of kozheed and cover with a lid from the Petri dish. Records of experiments are carried out daily within 72 hours. The experiments are carried out in 3 replicates at a temperature of 23 ± 1 ° C.

Analogously evaluate the duration of the action of the facility, planting insects every month (or, if necessary, through shorter intervals of time) for the same processed samples cloth.

For products that do not contain traditional insecticides, have a short or short-lived effect, including

when the insecticidal activity decreases after storage for
months, a similar method is used to evaluate the
(antifidant) action of the facility. In this case, the dried samples
4 × 4 cm tissue is weighed to the fourth digit,
put on them on 10 caterpillars of moths strictly specified above age,
Then the tissue samples along with the insects are transferred to glass
cups with a diameter of 3.5 cm, a height of 6 cm, which from above close
bryu.

The duration of the experiment is 14 days, after which
visually determine the number of bites and evaluate
damage to the tissue in points and weigh the tissue to the nearest
the fourth sign, determining the amount of tissue eaten
10 moth caterpillars.

Analogously, an evaluation of the anti-
the larvae of the skin-eater, but from the surface of the substrate and
the bottom of the cup with a brush collects all the excrement and also
weigh to the fourth digit.

Determination of residual effect on tissue or fur by method
accelerated aging. Storage of samples of processed wool
The cloth and fur semi-finished scrawl are held in closed
paper envelopes at elevated temperature (40 ° C) in a thermostat
(accelerated aging).

Calculation of the duration of action of the agent with accelerated
aging is calculated according to the "Interim Instruction I-42-2-82 for
work to determine the expiration dates of medicinal products.
means "on the basis of the method of accelerated aging" according to formulas 24, 25 and
26.

Method of studying insecticidal and ovicidal activity
specific means of fumigation type of action.

Assessment of the acute effect of agents in relation to moth caterpillars
are carried out in experimental vessels with a volume of 0.01-0.5 m^3 or

wardrobes volumes of 0.6-1 m 3 .

In the container, place the cloth (art. 3907), place the product in the required manner, 20 moth butterflies are introduced (in cages with size 10 × 10 cm, or in the case of small containers designed for estimates of funds in the form of small plates, in smaller cages), on 30 caterpillars on the food substrate (cloth size 10 × 10 cm), embedded in a canvas bag with a volume of 300 cm 3 , and 100 eggs per the same substrate. The experiments are carried out in 3 replicates at a temperature of 23 ± 1 ° C. Identifying the effectiveness of funds in relation to adults and larvae of the kozheed are carried out in similar containers, placing insects on the food substrate and then into a linen bag.

Accounting for the death of butterflies moths depending on the applied of the active substance is carried out after 24-72 hours, adults and larvae kozheedov - after 72 hours, eggs - after 10 days. Loss of caterpillars is evaluated daily for 3 days, in the absence of death or A low mortality rate after 14 days is estimated as mole-protective act.

Similarly, the study of the duration of action means, while not replacing the means and hang cloth and through certain time intervals in experimental tanks make insects.

Method for studying the insecticidal activity of fumigant type of action on the model object.

The insecticide-treated cardboard plates are placed in the middle part of glass vessels with a volume of 10 liters. In the vessels are placed Insect drinkers (small plastic container with wet cotton wool). Houseflies (imago 3-6-day-old age, without separation into half 100-150 pcs.) are discharged into the vessels, they are closed with a ligature, and fix the time of onset of paralysis with an interval of 5-15 minutes. at for 1 hour and further every 30 minutes. within 6-7 hours. After

lesions of 95% of insects in the test plate are removed from the vessel and
After 24 hours, the proportion of surviving individuals is taken into account. Evaluation of fumigation activity of insecticides is carried out by comparison indicators - CT_{50} and CT_{95} - time (min or hour), for which 50% (95%) of the insects in the experiment are in a state of knockdown and Indicator characterizing the reversibility of the state of paralysis - the proportion surviving individuals when recorded in 24 hours. Repeatability experiments three times. Experiments are carried out at a constant temperature air (23-25 ° C).

Performance indicators: acute action: knockdown 95% of individuals for 3-6 hours; Mortality in accounting at 24 hours is not less than 80%.

Method for evaluating the activity of agents of repellent type of action.

Preliminary assessment of the repellent effect is carried out in olfactometer, which is a box of plexiglas volume 0,04 m^3 , divided by a partition into compartments-traps. AT The experimental trap is placed as an attractive food substrate cloth of article 3907, on which the agent is applied (based on the rate of flow, which is 1/25 of the norm necessary for entering into a container with a volume of 1 m^3), and a control trap is placed

only said food substrate. Experiments are carried out in 3 replicates at a temperature of 23 ± 1 ° C. In the olfactometer, 50 butterflies are mothballed (1-2 days of age) and after 24 hours a repellent Action, the criterion of which is the number of butterflies moths and the number of eggs laid on the substrate in the experimental and control compartment. The deterrent action factor (RCD) for butterflies and moth eggs are calculated by the formula 12.

For testing in conditions close to in-kind, use wardrobes or other closed containers volume 0,5-1,0 m^3 , in which the required quantity is placed facilities. Experimental and control cabinets are flooded as imitation of clothes 1,5-2 m^2 of unapproved cloth of the article 3907. In all experimental volumes run through 50 moth butterflies 1-2 days of age. After 24 hours, inspect the cabinets and boxes and cloth hung in them. When examined, take into account

the number of live butterflies on cloth and the number of eggs laid by them.
Calculate the RCD value. To determine the duration
the action of the butterfly facility is started in experimental containers
at fixed intervals of time, without replacing in them the means and
cloth.

Performance indicators:

a) means of contact action: acute action - death
caterpillars of moths or larvae of goat-eaters after 72 hours 100%; durability
the residual effect is 4-6 months, the erosion of the tissue during
14 days - no more than 7 mg;

b) fumigation agents: acute action - death
Imago in 48 hours 100%; The duration of the residual action is not
less than 4 months;

c) repellant agents: RCD in the olfactometer through
24 hours - not less than 75%; the duration of the residual action is 1-4
months.

2.3.8. Methods for assessing insecticidal activity of agents for the destruction of wasps

Method for assessing the activity of insecticidal agents in aerosol packaging for the control of wasps indoors (when sprayed into the air).

Assessment of the activity of insecticidal agents in aerosol
packaging is carried out in the laboratory at a model facility -
Imago indoor flies of the insect culture. Into a 2 m_3 chamber
produce 300 flies. During the experiment, the temperature
air are maintained in the range of 22 ± 2 ° C to avoid unnecessary
the exciting or depressing effect of temperature on insects;
the relative humidity is maintained within 50-70%. When
study of each variant of the formulation of the means, the experiment is repeated
five times. The aerosol stream is directed to the chamber, while the flow rate
means should not exceed 1 g / m_3 . The expenditure of the funds is controlled
weighing the aerosol packaging before and after the contents are released.
Using a stopwatch, the time (T) of the lesion is 99%

insects in minutes and calculate the values of C_{15} (mg / m 3) - the value
the concentration of insecticide in the air, which causes a 99%

insects in 15 minutes, Q_{15} (mg / m^3) Is the amount of content

aerosol packaging, released from a balloon, causing damage

99% of insects in 15 minutes, and the value of KT_{50} (in min.) (Formulas 16, 17,

18, 22).

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Method for assessing the activity of insecticides used

way of irrigation, to fight the wasps in the nests.

Assessment of the activity of insecticides for the treatment of nests

os are carried out on imitations (models) of nests of wasps and on absorbent

surfaces of various types in the laboratory, using

As model objects of flies and red cockroaches. In the summer

experiment can be carried out in situ conditions directly on the

wasps in the nests.

a) Evaluation of the activity of insecticides in a special

Aerosol packaging on the models of the nests.

During the summer period, empty wasp nests are collected or

imitations of nests from five layers of tissue paper are made or

filter paper in 2 layers, reminiscent of the shape of the aspen

nests, a diameter of about 10 cm. Inside the nest are placed

100 imago room flies of laboratory culture without separation into

floor, the inlet is covered with gauze. Nest models are attached

adhesive tape (tape) to the wall at a height of 1.5 m from the floor level in

room with a volume of 10-15 m^3 . The processing of the nests is carried out from a distance

1,5-2 m at the rate of flow of 20-40 g / m^2, which corresponds to sputtering in

for 1-3 seconds. depending on the type of spraying device and

speed of evacuation of liquid from the package. Jet of liquid try

to direct into the hole of the socket model. Immediately after treatment

the tester must leave the room for 30 minutes. Then the room

the treated nests are placed in the gauze cages and

The flies are counted after 1 hour and 24 hours.

b) Evaluation of the duration of the residual effect on surfaces of various types of insecticides used

method of irrigation, to combat wasps.

In the event that the socket is located in the cavity of walls, roof and etc., it is not visible from the outside, and it is difficult to get to it, it is recommended Insecticide treated the surface around the cracks (cracks), through which the wasps penetrate inside the structure and fly back.

In the spray chamber, various test-surface: natural material of aspen nests in the size 10 × 10 cm, which is laid out in Petri dishes; plywood, slate in size 10 × 20 cm, cardboard or paper - 10 × 10 cm. The test surfaces are irrigated means from aerosol packaging from a distance of 20 cm at an angle of 45º at a rate of flow of 20 g / m_2 (or a working aqueous emulsion of the agent at the rate of flow 100-150 g / m_2). After 15 minutes. test surfaces remove from the chamber and they are imago imago flies (for 5 minutes) or red cockroaches (for 15 minutes), using standard exposition Nabokov-Laryukhin (D = 4 cm). Then the insects are transferred to dry clean plastic cups that are covered with gauze (flies) or plastic caps with apertures for aeration (cockroaches). Accounting deaths are carried out after 15 minutes. and 24 hours.

To evaluate the duration of the residual action of the test-surfaces should be stored at room temperature and periodically (after 2, 3, 5 days) to plant on them flies and cockroaches as described above. Records of death should be kept after 24 hours. If the number of dead insects after 24 hours does not exceed 50%, The study of the residual action is considered complete.

Method for assessing the activity of nonspecific insecticidal baits to fight the wasps indoors.

In laboratory conditions, when assessing nonspecific insecticide baits for the destruction of wasps as a test object use imago indoor flies. If necessary, research

Specific baits for wasps are carried out in field experiments
conditions.

Performance indicators:

a) insecticides in aerosol containers to combat
wasps in rooms (when sprayed into the air): acute action on the wasps
(flies - test object) C_{15} - not more than 10 mg / m^3 , Q_{15} - not more than 1000 mg / m^3,
CT_{50} - no more than 7 minutes;

b) insecticides in special aerosol containers
for the processing of nests: an acute action on the wasps-a test object-after 30
min. After processing the models of nests - not less than 90%, when flies are planted
(red cockroaches) on the absorbent test surface (after 15 min.
after treatment) - death of at least 100%; death in 24 hours - not less than
100%; duration of residual action - not less than 3 days;

b) insecticidal agents applied on the surface in the area
nests by irrigation: acute action on the wasps (flies is a test object)
when flies (red cockroaches) are planted on an absorbent surface
(15 minutes after treatment) - death of at least 100%; death through
24 hours - not less than 100%; The duration of the residual action is not less than
3 days;

c) nonspecific insecticidal baits: acute action on
os (flies - test object) - death in 24 hours not less than 80%, duration
the residual effect is 10-30 days.

2.4. Methods for studying the efficacy of acaricidal, insecticaricidal means and tissues containing insectoacaricides

Assessment of the effectiveness of acaricidal and insecticacaricidal
means should be carried out on active untrained females
type (species) of mites, in relation to which (s) will be given

recommendations for use. As a rule, these are species that have
the greatest epidemiological significance in a particular area.
For example, for the territory of the countries of the Eurasian Union, ticks of the genus Ixodes

are the main carriers of tick-borne pathogens
viral encephalitis, ixodid tick-borne borreliosis, etc.

Research, in the first place, should be carried out on the most

resistant to the effects of acaricides in the form of ticks. Among iksodovyh

Mites of the genus Ixodes are taiga mites. If necessary

studies are carried out on several types of ticks differing

biology, geographic distribution and sensitivity to

acaricides. In addition, it is possible to carry out experiments on males. When

the availability of sufficient justification is possible data dissemination,

obtained for the most sustainable species, on other species,

have similar or less resistance to acaricides.

As there are data on the similarity of biology and ecology of taiga and

forest mites and their close sensitivity to acaricides,

it is permissible to draw conclusions based on studies on I. persulcatus,

distribute to I. ricinus. However, for the recommendations of the application

against ticks of other genera (Dermacentor, Haemaphysalis, Hyalomma)

more research is needed. Since it is known that

Ticks of the genus Ixodes of insect culture are inferior in their

motive activity of the natural population, the study should

to conduct on mites collected in a natural biotope.

It is preferable to carry out the tests during the maximum period

activity of ticks in nature. The laboratory conditions are very

significantly differ from natural biotopes, where

use tools and protective clothing, therefore, based on

laboratory experiments can only do preliminary experiments, and

for final conclusions, it is necessary to evaluate the effectiveness of

means and protective properties of clothes in real conditions of use

(natural habitats) or conduct practical tests.

2.4.1. The method of studying the effectiveness of acaricidal and insecticocaricidal agents intended for processing
natural biotopes to destroy ixodid mites

Assessment of the effectiveness of funds designed to combat

iksodovyh mites in natural biotopes, carried out on

based on research on experimental sites in
natural biotopes (see section 2.8.4).

2.4.2. Methods for studying the efficacy of acaricidal and insecticocaricidal products intended for the processing of clothing for the purpose of individual protection of people against attacks of ixodids ticks, fleas and other arthropods

Method for studying the effectiveness of funds intended for

protection of people against attacks of ixodid ticks.

Such acaricidal and insecticacicidal agents permit

for processing clothes, equipment. They are represented

emulsion concentrates, aerosol and non-propellant

Packages, as well as special bars (pencils, crayons and

etc.).

Preparatory stage.

Of white cotton coarse calico, a control

and 3 experimental tests in the form of tapes measuring 70.0 · 10.0 cm. With a pencil on
Each test is done by marking: 5 cm from the bottom edge of the dough (place
pincers), 10 cm from the bottom edge (zero mark - the beginning
processed part of the dough) and then every 10 cm to a mark of 50 cm.

The control test should not come into contact with experienced tests or
hands contaminated with insectoacaricides! Experienced tests
are fixed on an absorbent surface (filter cloth

paper, etc.) measuring 80.0 · 40.0 cm, covering with polyethylene the lower
10 cm of tests. Then this surface with the tests is fixed vertically
and treated with a working solution of the emulsion concentrate or
aerosol in accordance with the recommended rate of discharge (usually for
aerosol and non-propellant aerosol packages - 20 g / m^2
surface). When evaluating the effectiveness of acaricidal bars
The strip is applied in accordance with the recommendations on
application at a distance of 10 cm from the bottom edge of the dough. After
processing tests are detached, dried and hung in
laboratory in the same controlled temperature conditions,
humidity, illumination. The tests begin in 1 day

after their preparation.

Evaluation of the effectiveness of funds intended for protection people from the attack of Ixodes tick-carriers of pathogens tick-borne encephalitis and ixodid tick-borne borreliosis are carried out in laboratory on female taiga tick I. persulcatus natural population. Use mites collected from vegetation no more than more than 1 day before the experiments and stored in wet bandages at a temperature of 10-15 ° C. The experiments are carried out at air temperature 22 ± 2ºC and relative humidity of 60-80%. Tests are fixed under angle of 70º to the horizon: use a special device for or the lower end of the dough is fixed with a plaster on table, and the upper one - on the wall adjacent to the table.

Ticks one copy are placed 5 cm below the zero mark and observe their movement up the fabric,

additionally stimulating them with the finger of the observer who is holding at a distance of 0.5 cm from the proboscis (gnatosome). Record the time from The moment when the mite crosses the zero mark before it falls off the test, which corresponds to the onset of paralysis of the limbs (knockdown). At the same time, the maximum lifting height each tick from the zero mark. The missing ticks are placed in 70% solution of ethyl alcohol for the purpose of fixing them or into a test tube with high (about 90%) humidity for checking the reversibility of knockdown. Knockdown is recognized as reversible if the tongs are able to hold and crawl up the fabric after 15, 30, 60 min. or 1 day after registration of knockdown. Experience is repeated with no less than 10 females per each of the three test runs. The mean value time of the onset of knockdown in minutes (CT_{cf}), average The maximum height of lifting of each tick in the test in centimeters (MV_{cp}).

Between the tests, the tests are suspended in the laboratory at room temperature 22 ± 2ºC, relative humidity of 60-70% and natural light.

The determination of the suction rate index (ICP) is carried out according to

item 2.2.5. The time of contact of the mites with the processed part of the test runs should equal ½ KT $_{cp}$.

To establish the duration of the protective action,

repeated tests before the decrease is below the normative.

The frequency of testing is determined by the testing laboratory (center).

The duration of the protective action and indication of the reversibility of knockdown

ticks are given in the operating documentation. For funds,

containing insecticacaricides, after tests on female taiga

tick indicate the purpose: to protect against ixodid

mites carriers of tick-borne encephalitis and

borreliosis. Indication of vectors of other infections (Crimean

hemorrhagic fever, rickettsiosis, etc.) is possible only after

tests on species of ticks

carriers of these infections. In the text of a label (for a life) specify:

"Violation of the rules of conduct and the way the

lead to the sucking of ticks. Be careful!"

Performance indicators: CT $_{rms}$ - no more than 5 minutes, CF $_{cp}$ - not more than 50 cm, ISP - not more than 1.1.

Method for studying the effectiveness of insecticacaricidal agents,

intended to protect people from flea attacks.

Studies are performed in the laboratory on hungry rat

fleas of Henopsylla cheopis sensitive insect culture.

Experiments are carried out at an air temperature of 25 ± 2 ° C and a relative humidity of 60-70%.

Preparatory stage.

Of pure white cotton coarse calico

control test in the form of a strip measuring 50.0 · 1.5 cm. Control

The test should not come into contact with experienced tests or hands,

contaminated with insecticacaricide! For the production of test runs

The tissue of size 60.0 · 10.0 cm is fixed on an absorbent surface

(cloth, filter paper, etc.). Then this surface with the test

fixed vertically and treated with a working solution

emulsion concentrate or aerosol in accordance with the recommended

the rate of consumption (usually for aerosol and non-propellant aerosol packages - 20 g / m 2). After completely drying

treated tissue (1 hour) from its different sites are cut out 3 experienced

test in the form of strips measuring 50.0 · 1.5 cm. Strips with a pencil mark the length of the test (5, 10, 15, 20, etc. cm).

Evaluation of the activity of fleas of laboratory culture is carried out immediately before assessing the effectiveness of the remedy, using control test.

To the center of the inner surface of the glass cover from the cup Petri (diameter about 9 cm) glue the hook to which the control test is suspended. In a glass measuring cylinder 70 cm high and 8 cm in diameter, 30 fleas were placed. The cylinder is placed in center of the pelvis with high sides (at least 20 cm).

Cover the cylinder with a lid, placing the control test in the center cylinder so that the bottom edge of the test is 1 cm below the bottom. Turn on the stopwatch. Fleas jump on the control test, move it up and reach the lid. Indicator sufficient activity of fleas of insect culture is staying in 5 minutes. on the control test and cover more than 90% fleas placed in the cylinder. After checking the activity of fleas collected by the exhauster and destroyed (boiling water, ether, etc.). Pelvis serves as an additional insurance against flea dispersion, since his fleas can not get out.

Evaluation of the effectiveness of the means begin after the experiment with control test. A new flea group of 30 individuals of laboratory culture, then instead of a control test put the first test in the cylinder, include a stopwatch and observe the behavior of fleas. Fleas should hop on Experimental test and fall off from it to the bottom of the cylinder as a result the onset of knockdown (the state of paralysis of arthropods, expressing itself for fleas in the inability to bounce and crawl

up). The number of fleas on the test is recorded in 5 minutes. after the beginning of the experiment (KB_5) and the height of the rise of 3 fleas, risen in the test above other individuals (MB). After graduation Each experiment of fleas is collected by an exhauster and placed through a funnel in glass test tubes to account for the state of insects (mobility, mortality) in 15 minutes, 1 hour and 1 day after the start of the experiment. For these the results are judged on the reversibility of knockdown. Then these fleas destroy. Each experiment is conducted on 3 tests and three groups of fleas (repetition), calculate the average performance indicators (KB_5 - the average number of fleas on the test after 5 minutes. after the beginning of the experiment and MV_{cp} is the average maximum height of the flea rise).

Between the tests, the tests are suspended in the laboratory at room temperature 22 ± 2ºC, relative humidity 50-70% and natural light. The tests begin in 1 day after their preparation and repeat to establish the duration Protective action after 7, 14 or more days before the decrease in indicators below the normative. The test frequency is determined by the test laboratory (center). Reversibility of knockdown and duration insecticidal action of the agent is indicated in the description of the properties of this facility.

Performance indicators: KB_5 - no more than 3 copies; MV_{cp} - not more than 20 cm.

Method for studying the effectiveness of insecticacaricidal agents, designed to protect people from attacks by mosquitoes, midges and other flying bloodsucking insects.

Such insecticacaricidal agents are allowed for treatment clothes, and they are represented by concentrates of emulsions, aerosol and non-propellant packages. Research

effectiveness of such insecticacaricide only in natural conditions (see Section 2.8.4.).

2.4.3. Methods for studying the protective properties of tissues containing insectoctacaricide

Method for studying the protective properties of tissues containing insectoacaricides and intended to protect people from attack Ixodes ticks.

Preparatory stage.

Of white cotton coarse calico, a control a test in the form of a tape measuring 70.0 · 10.0 cm. A control test should not contact experienced tests or hands that are contaminated insectoacaricides! For the preparation of experimental tests from the studied Fabric area of 2 m^2 is cut from different places 3 dough in the form of tapes size 70.0 · 10.0 cm. On the bottom 10 cm of the test tests, paste clean cotton cloth. It is recommended that A glue stick or other glue, after making sure that its smell after airing does not cause scarring of ticks. Pencil on each test make marks: 5 cm from the bottom edge test (the place of mite replanting), 10 cm from the bottom edge (zero mark - beginning of the test tissue) and then every 10 cm to the mark 50 cm.

Evaluation of the protective properties of the tissue is carried out analogously to the method study the effectiveness of means designed to protect people from the attack of ixodid ticks (see paragraph 2.4.2 above).

Performance indicators: CT_{rms} - no more than 5 minutes; MV_{cp} - not more than 50 cm; ISP - not more than 1.1.

Method for studying the protective properties of tissues containing insectoacaricides and intended to protect people from attack fleas.

Preparatory stage.

From pure white cotton coarse calico make control test in the form of a strip measuring 50.0 · 1.5 cm. Control The test should not come into contact with experienced tests or hands, contaminated with insecticacaricide! From the studied tissue containing

insectoacaricides, an area of 1 m^2 is cut from different places 3 experienced test in the form of strips measuring 50.0 · 1.5 cm. For pencil tests mark marks of length (5, 10, 15, 20, etc. cm).

Evaluation of flea activity and protective properties of the tissue is carried out similarly methods study effectiveness of means, designed to protect people from attacking fleas (see paragraph 2.4.2 above).

Performance indicators: KB_5 - no more than 3 copies; MV_{cp} - not more than 20 cm.

Method for studying the protective properties of tissues containing insectoacaricides and intended to protect people from attack mosquitoes, midges and other flying insects.

Such research can be carried out only in natural conditions, where there is an unlimited number of bloodsucking flying insects attacking a person for bloodsucking (see 2.8.7.).

2.4.4. Methods for assessing the activity of acaricidal agents, intended for the destruction of blood-sucking gas-mites in premises

As a biological material in laboratory experiments use hungry sexually mature individuals rat tick O. bacoti. When selecting ticks in an experiment a random sampling method is used. Subjects and control Mites should be of the same age. Ticks contain optimum conditions for them in the thermostat at air temperature 25-26 ° C and relative air humidity of 80%. Every experience accompanied by a control option.

Evaluation of the effectiveness of funds intended for processing surfaces in the premises to destroy bloodsucking

gamma mites, carried out depending on the formulation facilities.

The method of replanting the acaricide-treated test surface (for studies of the activity of liquid and powder forms).

The acaricidal effect of the agent is evaluated in laboratory conditions on surfaces of two types - absorbent (plywood) and non-absorbent (glass). To prevent ticks from spreading experiment of the edge of glass plates and pieces of plywood standard 20 × 10 cm in size are treated with repellents effective in O. bacoti (20% solutions of dimethyl phthalate or acrepe), or during the contact time the mites do not allow them to leave test surfaces and returned back using a thin brush.

Acute acaricidal act define the method forced contact of ticks with freshly prepared surface (after drying test surfaces). Contact time

is 5 minutes. After this, the mites are transferred to clean glass tubes with cotton-gauze stoppers and placed in a thermostat. Accounting the affected individuals are carried through 24, 48, 72 hours.

Residual acaricidal action of the agent is determined in the same way, for 5 min. to processed test surfaces on days 3, 7, 14 and more until the end of the acaricidal actions. After exposure, the mites are transferred to clean containers and is placed in a thermostat. Accounting for dead mites is carried out through 24, 48, 72 hours after contact with the treated surface.
The glass plates and plywood used in the experiment are stored in vertical position at room temperature.

Method for assessing the activity of agents in aerosol and non-propellant packaging.

Evaluation of effectiveness of means at aerosol and non-propellant packings with respect to rat mites are carried out in accordance with the standard methodology set out in paragraph 2.3.1. by testing of aerosol packaging for destruction flightless insects. In order to determine the acute effect

means of rat mites are placed in Petri dishes (not less than
30 individuals per each), whose edges are lubricated with petroleum jelly or 20%
solution of repellant (dimethyl phthalate, acrepe) to avoid
spreading of ticks. Petri dishes are placed at 5 points in the chamber and
then treated with aerosol or non-propellant
packaging.

In 10 minutes. After application of the preparation, Petri dishes are removed
from the chamber and transplant the mites into clean test tubes that are
is placed in a thermostat. The mortality of mites is counted at 24, 48,
72 hours.

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The residual action is determined by placing the mites on
treated with aerosol or non-propellant packaging
test-surfaces (Section 2.3.1.).

Performance indicators: acaricidal agents for combating
gamazovymi mites in the premises: acute action - death of mites
after 24 hours, 100%, the residual effect - the mite death in 24 hours
when replanting for 3 days on a non-absorbent surface, not less than
80%.

2.4.5. Methods for assessing the activity of funds intended for fighting
with acariform ticks
(scabies and house dust mites)

Methods for assessing the activity of agents (scabicides),
intended for the treatment of surfaces in the premises, clothing,
linen, etc. with the aim of destroying scabies.

Evaluation of the effectiveness of funds intended for processing
surfaces in the premises, clothes, linen, etc. with the aim of destroying
scabies mites are carried out in vitro on a model object - the ear
rabbit mite P. cuniculi insect culture.

Depending on the formulation of the product evaluation
Scabicidal activity is carried out by three methods described below.
For a concentrated agent, the sample is vigorously shaken and
on the scales a sample is taken to prepare a series of workers
concentrations. By dilution with water, the necessary

concentration. Ready-made formulations are tested without
breeding.

Method of immersion for the study of liquid formulations
scabicides (emulsions, suspensions, solutions, etc.).

Female ticks of 10 individuals are placed on a circle of filtering paper, folded angle in the form of a "pound", immerse in the studied working liquid for 1 min. Then the mites are transferred to a clean a test tube with filter paper embedded in it. After 24 hours determine the number of affected ticks and calculate the percentage defeat. Experiments are performed at room temperature in three replicates. As a control variant, untreated ticks placed in tubes similar to way.

The method of irrigation (spraying) for assessing the scabicidal activity of means.

Female ticks of 10 individuals are placed in Petri dishes on absorbent (filter paper, coarse calico) surface, after which Petri dishes with mites are randomly housed in a chamber in five points and treated with aerosol or non-propellant packaging, or aqueous working solutions various preparative forms with the help of equipment of the "Kvazar" type or "Rosinka" from a height of 20 cm, directing the jet means at an angle of 45 ° to the bottom of the camera. The temperature in the chamber during the experiments should be constant 22 ± 2 ° C, relative humidity - not less than 60%. On the edges cups should be applied a strip of petroleum jelly to prevent spreading of ticks. In 10 minutes. Petri dishes are removed from the chamber, ticks are transplanted into clean test tubes and counted for death by 24 hours, counting the percentage of affected individuals. Experiments are conducted at room temperature in triplicate. As a control

use untreated mites placed in test tubes
the same way.

The method of replanting the surface treated with a scabicide.

The method is used to study the efficiency of dusts and other powdery forms, as well as to study the duration residual action. Ticks of mites for 10 individuals are planted on treated with a non-absorbent (glass) and absorbent (filter paper, coarse calico) surface for 15 minutes.

To establish the residual action of the medium, samples surfaces - plates of glass and filter paper or calico size 10 × 20 cm placed in the chambers at least 5 pieces each and is treated as described above. Test-surfaces are left for the subsequent study of the residual action of the agent.

After exposure, the mites are transplanted into clean test tubes and account for death in 24 hours, counting the percentage of affected individuals. Experiments are carried out at room temperature in three replicates. As a control, untreated
Ticks placed in tubes in the same way. Determine effective concentrations and rate of use of the funds that provide sufficient quality (uniformity) of application to different types surfaces with ensuring scabicidal effectiveness.

The residual effect of the deposits of the agent is determined after treatment for 1, 3, 5, 7 days or more by contacting ticks with treated surfaces for 15 minutes. in the exposition.
After contact with the treated tests, the specimens are transferred to pure test tubes and record their condition after 24 hours.

Performance indicators: acute action - death of mites in 24 hours 100%; residual action - death of mites when replanting

for 3 days on the non-absorbent surface, when recorded in 24 hours, not less than 80%.

Methods for assessing the activity of agents (acaricides),

intended for the destruction of house dust mites.

As a biological material in laboratory

experiments use the seeds of home dust mites.

Pyroglyphidae: D. pteronyssinus and / or D. farinae. Experimental and

Control tongs should be of the same type. Ticks contain

stable, optimal for their development conditions, in thermostats with

at a temperature of 25-26 ° C in glass densely closed desiccators with

relative air humidity of 80%. Food ration of mites

It should contain all the necessary components for their normal

life activity.

In laboratory conditions, the assessment of acaricidal activity

means designed to combat house dust mites,

spend on an absorbent fabric surface (coarse calico). Dimensions and

the shape of pieces of coarse calico used as a test surface,

corresponds to the shape and size of the bottom of the vessel (small, small

glass cups, etc.), which contain ticks during

experiments. The edges of each vessel with house dust mites

pre-pasted with adhesive tape to avoid spillage

mites (the "hunting belt"). Each experience includes at least

3 replicates, in each of which at least 20 ticks are used, and

accompanied by control.

The method used to assess the effectiveness of funds,

intended for the destruction of house dust mites, choose

depending on the form of the preparation.

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Method for assessing the activity of agents in aerosol and

non-propellant packaging.

To assess the effectiveness of agents in aerosol and

non-propellant packages use the standard method

spraying of arthropods with a chamber of 1 m^3 volume . At five points

Place Petri dishes with mites placed directly on the

coarse calico. At the same time, test surfaces are also placed in the chamber -

pieces of fabric (coarse calico) - at five different points to study the duration of the residual action of the deposited funds. Ticks and coarse calico irrigate the product from a distance of 20-30 cm at an angle of 45 °.

In 10 minutes. After irrigation, the mites are transferred to clean dishes and placed in desiccators. The death of ticks is taken into account through certain time intervals (10 minutes, 24, 48 hours after processing). Defeated believe dead and irreversible paralyzed ticks.

The processed test surfaces (coarse calico) are stored in a suspended position at room temperature. On days 1 and 3 after treatment for determining the duration of residual action of sediments aerosol on them are planted with ticks with the addition of feed substrate and left for further observation. Death accounts mites spend for 5-14 days.

The method of replanting ticks on the treated test surface (for studies of the activity of liquid forms).

Treated tissue samples (coarse calico) are placed in a glass a small vessel. After complete drying on coarse calipers mites and add a fodder substrate. Inclusion of affected mites is carried out after 24, 48, 72 hours.

Performance indicators: acute action - death of mites after 24 hours not less than 100%.

The method for treating the medium for culturing ticks (to study active substances, liquid preparations).

The culture medium of the mites is treated with acaricidal activity. After complete evaporation of the solvent in substrate is placed with 30-50 mites. Accounting for live animals is carried out through 24, 72 hours, then once in 7 days for 3 weeks.

Performance indicators: mite death in 3 weeks is not less than 100%.

2.4.6. Methods of studying systemic and contact action insectoctacaricides on a warm-blooded animal

Method for assessing the activity of insect-rodenticidal agents. AT
experiments use arthropods from sensitive insect
cultures - rat flea Xenopsylla cheopis and rat mites
Ornithonyssus bacoti. Use non-absorbed imago fleas
1-3 weeks of age and adult hungry ticks without separation by
sex. As a feeder, white laboratory mice
with a mass of 30-40 g. When selecting fleas and ticks for experiments
observe the method of random sampling.

Evaluation of the systemic action of new active substances (DV)
insecticides are carried out in a dose range of 1-30 mg / kg in two steps:

a) Forced administration of insecticacaricide to white mice
and the range of active doses for
1-7 days;

b) direct testing of finished baits containing
Insectoacaricide and rodenticide for 1-3 days depending on the

chemical structure of the Far East and the type of experimental arthropod,
containing only insecticacaricide up to 8-15 days.

The method of studying the systemic action of an insecticaricide according to
relation to ectoparasites of rodents (laboratory culture of fleas and
blood sucking gamasid mites) with his compulsory
oral single administration to white mice.

White laboratory mice, average weight 30 g, starving in
2-3 hours, once orally administered 0.5 ml of water
emulsions of insecticide in a dose of 1, 10 and 30 mg / kg (0.03, 0.3 and 0.9 mg / individual,
respectively). After 1, 2 and 3 days after the insecticide was administered on
these mice are planted by hungry arthropods. In this case, separately
conduct a series of experiments with fleas and ticks. For
prevention of combing and eating ectoparasites in mice,
animals are placed in a cage 3 × 3 × 8 cm in size from metal
Grids with 1 × 1 cm cells that are placed on Petri dishes with nested
paper filters. Further, these Petri dishes are placed in large
plastic containers with high walls that allow
keep all ticks and fleas that left the feeder after

power supply. When using ticks in the experiment, to prevent them run out, the walls of the tanks are smeared with petrolatum along the top edge. The experiments are carried out at room temperature 22-25 ° C. Each experience is accompanied by control options.

Fleas.

In mice, 30 imago fleas are implanted for 120 minutes. Then fleas collect from a plastic container, and also comb the remaining insects with animals plastics frequent crest. Take into account share of impregnated fleas. The collected fleas are placed in test tubes and

is removed to a thermostat (28 ° C, relative air humidity 70%). The mortality of the impregnated fleas is recorded after 24 hours.

The gas mites.

In mice, 30-40 adult hungry mites are planted O.bacoti. After 4-5 hours, the mites were collected and they were incubated in wet tubes, made according to the procedure E.N. Nelzina. The mortality of mites is counted after 24 hours.

Method for evaluating the systemic effect of baits containing insectoacaricide, in relation to rodent ectoparasites (laboratory culture of fleas and blood-sucking gamasid mites).

The effectiveness of baits is determined by the daily for three days of ticks and fleas on mice, constantly feeding bait containing an insectoacaricide.

Evaluation of the feeding behavior of white mice in the experiment.

Evaluation of bait consumption is carried out by daily weighing feeds on electronic laboratory scales with accuracy weighing 0.005 g. Feed consumption is expressed in grams per individual per day. Calculate the dose of mouse absorbed DV (mg / kg weight of an animal per day) based on its content in feed (% DV) and established bait eatability.

The definition of systemic action with respect to bloodsucking gamma mites and fleas containing insecticacaricide bait at feeding it to white mice.

Arthropods (separately fleas and ticks) daily for

1-7 days of experiment are placed on mice, constantly
feeding bait with an insectoacaricide.

Performance indicators.

When administering solutions forcedly by oral white
mice: mortality of fleas and blood-sucking
feeding on 1, 2, 3 days after the introduction of insecticide is not
less than 80% if registered after 24 hours; when feeding poisoned
bait: eating at least 80% of that in the control
variant (untreated bait); mortality of fleas and ticks
feeding on mice for 3 days at least 80% when recorded in 24 hours.

Method for studying the duration of contact action
insecticides on rat fleas when tested on white mice.

In laboratory studies are performed on non-absorbed imago
fleas 1-3 weeks old without sex division of the rat flea
Xenopsylla cheopis from a sensitive insect culture. As
the feeder is used in white laboratory mice weighing 30-40 g.
When sampling insects for experiments, observe the method of random
of the sample.

When carrying out research cause a drug on
experimental animals in a wide range Doses:
1, 10, 50, 100, 200 mg LW / kg of animal weight.

Recommended schemes for conducting experiments to study
proposed for testing funds based on various DV:

a) for WCF - the definition of the duration of the action should be
conduct at 2, 24, 48, 72 and 168 hours after application to the animal;

b) for insecticides with a pronounced residual
action - pyrethroids, phenylpyrazoles, neonicotinoids - fleas
should be re-injected weekly until the indicators decrease
insecticidal action below 50%.

Flea care from an animal can be caused by various reasons:

1) the end of the supply (in the control version for 120 min.
up to 20% of insects usually leave the animal);

2) repellent effect of insecticide;

3) paralysis of insects, due to which fleas fall from the animal.

Not being able to distinguish the reasons why fleas
leave the provider, the term "scaring away" is
in quotation marks.

Method for studying the duration of residual action
insecticides on a rat flea.

White laboratory mice with an average mass of 30 g are placed in
cages measuring 3 × 3 × 8 cm from a metal grid with 1 × 1 cm cells and
treated with 0.1 ml insecticide, evenly applying drops on top of
both sides of the animal's spine. Required concentrations
insecticide are obtained by sequential dilution of preparative
forms of insecticides by propylene glycol ether. In the experiments,
doses of 1-100 mg of insecticide DV per kg of animal weight (0.03-3.0 mg of DW
on the mouse, respectively). The cages are put in Petri dishes with nested
paper filters and placed in large plastic containers
with high walls. 2 hours after application of the insecticide
are planted on the mice of adult fleas (at least 30 insects per
repetition). Within 120 min. take into account the "frightening" action
(leaving fleas from the body of the animal) with an interval of 15-30 minutes. Then fleas
collect from a plastic container, and also comb the remaining
insects with animals with a frequent plastic comb, which
use once. Consider the proportion of impregnated fleas, fleas
who left the feeder and left on the body of the animal.
The collected fleas are placed in test tubes and put into a thermostat (28 ° C,
relative air humidity of 70%). The death of insects is taken into account

in 24 hours. The residual effect of the test substances is determined

by the same method, using previously treated mice, each of which are contained in a separate cell. Fleas are planted after 1, 3, 7 days and then weekly until the termination of the facility (term observations of 30-35 days). Experiments are carried out in two or three repeat, using at least 30 insects in each. Every experience two control options: flea processed solvent and untreated animal. The experiments are carried out at room temperature (22-25 ° C).

For comparisons the results use indicators The duration of the "deterrent" (FROM $_{50}$ and OT $_{95}$ - time, during of which the treated animal leaves 50% and 95% of the fleas, respectively) and insecticidal action (CT $_{50}$ and CT $_{95}$ - during which 50% and 95% of the fleas die respectively experiment), expressed in days.

2.5. Methods for determining the effectiveness of repellent agents

2.5.1. Methods for assessing the effectiveness of repellent products and products in insects

The method of assessing the activity of repellent means, intended for application to the skin (creams, lotions, emulsions, napkins, pencils, aerosols).

To tests are allowed only means having confirmation of the safety of their use on the skin of people. In the experiments use the hungry female Ae.aegypti of the insect culture in aged 8-10 days, who received carbohydrate nutrition. In the reticulum size 30 × 30 × 30 cm produce 50 ± 5 females. Such conditions of experience correspond to high numbers of mosquitoes. Tests of repellent

funds begin in 30 minutes. after the start of mosquitoes. Experiences conducted at an air temperature of 25 ± 2 ° C, relative humidity 60-70%, with diffuse lighting, avoiding direct sunlight.
Tests should be conducted from 9 to 14 hours.

To confirm the activity of mosquitoes, the cage is placed bare forearm of the tester, protecting the rubber hand

glove. Record the plantings and mosquito bites for 30 seconds.
The activity of mosquitoes is considered satisfactory, if for this
a period of at least 10 plantings and 3 bites. After that
the test substance is applied to the entire surface of the naked
forearm test in the rate of consumption of 0.1 ml (g) per 100 cm 2 of skin.
The forearm of the second hand is treated in the same rate of application
the benchmark of the proposed efficiency category. The standard of the highest
efficiency category is a 30% solution of DETA in ethyl
alcohol, the standard of the first category - 20% solution of DETA, the standard
the second category - 10% solution of DETA, the standard of the third category -
5% solution of DETA, the standard of the fourth category - 3% solution of DETA.
Both hands are placed in a cage with mosquitoes for 3 minutes. and register the number
plantings and mosquito bites. Immediately after application, repellent
means, as a rule, ensure the repelling of all insects, then
there is a RCD of 100%. In order to determine the duration
repellent action (DRD), repeat the experiment through
every 30 minutes. until 3 or more are registered
bites in 3 minutes. tests. Repeated testing in each cage
can be conducted no earlier than an hour after the previous one. Each
the test is carried out by at least three testers in at least three
replicates (a total of 9 replicates). The mean value

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DRD. The studied funds are divided by efficiency categories
repellent means, focusing on indicators.

To the highest category of efficiency is the means, for which
ДРД is 4 and more hours, to 1 category - 3 and more to 4,
to 2 categories - 2 or more to 3, to 3 categories - 1 or more to 2,
to 4 categories - 1 or more to 2 (with a low number of mosquitoes).

If it is assumed that the repellent agent is 4
efficiency category, then its tests are
certain changes in the methodology of experiments leading to a reduction in
aggressiveness of mosquitoes: in a cage measuring 50 × 50 × 50 cm produced
20 ± 2 females. All other conditions of the experiment remain the same.

Performance indicators.

CODE - 100%, duration of repellent action in hours in accordance with the categories of effectiveness: the highest category - 4 and more (the label indicates the protective effect of insects more than 4 hours), 1 category - 3 or more to 4 (in the label indicate protective action from insects up to 4 hours), 2 category - 2 or more to 3 (the label indicates the protective effect of insects up to 3 hours), 3 category - 1 or more to 2 (in the label indicate a protective action from insects up to 2 hours), 4 category - 1 or more to 2 (in the label indicate the protective effect of insects up to 2 hours at low number of mosquitoes).

The full spectrum of the repellent action of the agent is determined by based on the study of the effectiveness of the agent in full-scale conditions or on the basis of literature data.

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The method of assessing the activity of repellents in the form of aerosols, intended for protection against Dipterous insects when applied on clothes.

Apply the method of processed sleeves. Sleeves are prepared of cotton coarse calico (fabric sleeves should be pretty tight adjoin to the forearm of the test) and treat the tissue with aerosol at a rate of 20 g / m_2 . The repellent efficiency test is carried out the first time after the fabric has dried completely (after about 1 hour) and then after 1, 2, 3, 5 and further days. In between tests

The sleeves are suspended in the laboratory at room temperature 22 ± 2 ° C, relative humidity of 60-70% and natural light. When tests the treated sleeve is worn on the forearm of the tester (a hand in a rubber glove) and put the hand in a mosquito net similar to the procedure described above. The end of the repellent Actions are considered to be a day when 3 or more bites are recorded The mosquito is through the fabric of the sleeve for 10 min. tests.

Performance indicators:

CODE - 100%, DRD in days in accordance with categories efficiency: the highest category - 20 or more days (in the label the protective action from insects is indicated for more than 20 days); 1 category - 10 or more to 20 days (the label specifies a protective action from insects up to 20 days), 2 category - 5 and more up to 10 (the label indicates the protective effect of insects up to 10 days), 3 category - 3 or more to 5 (the label indicates the protective action from insects to 5 days).

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Methods for assessing the activity of repellent means, intended for protection from ants.

Assessment of the effectiveness of funds designed to protect against ants, carry out on the basis of research in natural biotopes (see Section 2.8.7).

Method for studying the protective properties of articles containing repellents and intended to protect people from attack mosquitoes.

The method is designed to determine the protective properties repellent bracelets, stickers and other products that have repellent properties for blood-sucking mosquitoes and intended to protect people from their attacks.

Studies are carried out in laboratory conditions in relation to Insect culture of mosquitoes Aedes aegypti. Derived in the insect females of 20 ± 2 individuals aged 8-10 days kept on carbohydrate feeding, is placed in a mesh size of 50 × 50 × 50 cm. Such the setting of the experiment is an imitation of a low number of mosquitoes, Because it is known that the density of mosquitoes in the cage determines their aggressiveness.

If the product (for example, a sticker) is intended for sticking on clothes, a test piece is glued to the strip of calico is wider than the sample, not less than 1 cm.

The experiments are carried out from 9 to 14 hours at an air temperature of 25 ± 2 ° C, relative humidity of 60-70% and natural light.

Before starting the tests, put the forearm in the cage (brush hands are protected with a rubber glove) and carry out a control record: in for 1 min. take into account the number of mosquito bites by the forearm.

The activity of mosquitoes is considered sufficient, if for the control account registered at least 3 bites when simulating low numbers.

For tests, the bracelet is worn on the wrist, a strip of coarse calico with glued product is fixed on the forearm near the elbow.
Use cages with mosquitoes that were not previously in the experiments. When conducting tests, the test person places a forearm with a bracelet or fixed by another sample of the product in the tank, and spend 3 minutes counts the number of mosquito bites by the forearm and the test brush.

Coefficient of protective action (CPD $_{mosquitoes}$ in percentages) is calculated by the formula 19, where A is the number of mosquito bites on the arm without a product (control) in terms of 3 minutes (duration experience); B - the number of mosquito bites on the arm with the product (experience).

Each experiment is performed in 9 replicates (3 testers per 3 replicates) and calculate the average index of protective action (CPD $_{mosquitoes}$).

Experiments begin in 15 minutes. after removing the product from the packaging. To determine the duration of the protective action repeat tests before reduction of CPD $_{mosquitoes}$ below normative. The test frequency is determined by the test laboratory (center) based on the declared duration of the protective actions. Between the experiments, the products are suspended in a ventilated room, at a temperature of 22 ± 2 ° C, relative humidity of 60-70% and natural light. For bracelets, stickers and other similar products showed DRC $_{mosquitoes}$ less than 90%, the label indicates purpose "to reduce the number of mosquito bites." For products, showing CPD $_{mosquitoes}$ equal to or greater than 90%, the label indicates "for protection against mosquito bites ". Duration of protective action

indicate in the label.

Performance indicator: CPD $_{mosquitoes}$ - not less than 30%.

2.5.2. Methods for assessing the effectiveness of repellents for protection from ixodid ticks

The method of assessing the activity of repellents in the form of aerosols, intended to protect against ixodid mites when applied to clothes. A test is used of cotton coarse calico - tape 10 · 70 cm, which is fixed to an absorbent surface (fabric, filter paper, etc.) in the size of 0.5 m^2, covering with polyethylene the lower 10 cm. Then this surface with the dough is fixed vertically and treated with aerosol in accordance with the recommended standard flow rate, typically 20 g / m^2 surface area.

After processing, the tests are detached, dried and hung in a laboratory under the same controlled temperature conditions, humidity, illumination. The experiments are carried out on the day of treatment. Tests fixed at an angle of 70º to the horizon. Ticks one by one are placed on 5 cm below the zero mark and observe their movement upwards tissue, further stimulating them with the finger of an observer who keep at a distance of 0.5 cm from the proboscis (hypostoma). Record number of mites crawling the segment treated with repellent means, length 50 cm. On the control test, as a rule, all mites creep the marked zone. After testing, the mites are placed in 70% solution of ethyl alcohol for the purpose of their preservation or continue further observation of them. Experience is spent no less than with 30 females. Calculate the CODE, which in this case is equal to the fraction mites (in percent), not crawled the treated area from the number ticks in the experiment. The results of the experiments are interpreted in comparison with the results of the benchmark tests. For determining

the duration of the repellent effect of the test is repeated daily as long as the COD remains equal to or above 90%. Because the repellents do not protect completely from ticks - carriers of pathogens of dangerous diseases, in the text of the label (for everyday life) indicate "The facility provides incomplete protection from ticks. Be careful!"

Performance indicators: RCD - 95% or more, DDD - 3 or more days.

Method for the evaluation of acaricidal-repellent agents in the form of aerosols intended to protect against ixodid mites when applied to clothes.

Assessment of acaricidally-repellent means is carried out on two directions, as an acaricidal agent and as repellent. Test prepared in accordance with 2.4.2. The study of effectiveness is carried out according to para. 2.4.2., 2.5.2. The indices of CT_{scales}, CF_{cp}, ICP, COD and DDD are determined .

2.5.3. Method for assessing the activity of repellent means in relation to gamaside mites

Assessment of the effectiveness of repellents in relation to O. bacoti is carried out using a technique developed for ixodid ticks, and a negative locomotor reaction is evaluated mite on the boundary of the chemical barrier, which is a Repellent applied to the substrate on the route of the ticks. Way is determined by the negative geotropism of the hungry O. bacoti ticks, backed up by an attractive extraction factor (the hand of the experimenter). A deterrent effect is investigated at 1, 3 and 5 day after treatment. Experience with each connection is not carried out

less than 3 replicates, using at least 30 ticks in each. AT experience use of hungry female rat tick.

To assess the repellent activity of substances, calculate coefficient of repelling action (RCD) by the formula 12.

The duration of the repellent action (DRD) is estimated as time

(in days), during which the zone treated with a repellent,
pass no more than 30% of ticks.

2.6. Methods for determining the activity of funds based on regulators
development of insects

The Insect Regulators Group (PPH) unites
connections, which are the mechanism of action of analogues of natural
insect hormones: juvenile (Ayug or Juvenoid), linoleic
(LH or ecdysoids); neurohormones, etc. This group also includes
chemical compounds that are not analogous in structure
natural hormones, but causing hormones in insects
effects. These include inhibitors of chitin synthesis (ISX): chloro- and
fluorinated derivatives urea (diflubenzuron, triflumuron,
hexaflumuron, etc.). From the group of analogues of the juvenile hormone in Russia
Methoprene, hydroprene and pyriproxyfen are the most well known. For
Characteristics of the activity of AYG and ISX are used
effective concentrations and doses, causing morphogenetic or
other changes in 50, 95 or 99% of bioobjects.

The mechanism of action of AUG is that the introduction of
exogenous analogue in the period when the true hormone titer in
the insect organism is minimal (the "critical period"), causes
effects that are absent in the normal passage of metamorphosis.
Since these compounds are analogues of a natural hormone,

the body reacts to their appearance (presence) by education
intermediate, giant, strongly melanized
larvae, underdeveloped pupae with developed head of imago, etc.

Chitin synthesis inhibitors are compounds that
inhibit the process of glucose formation necessary for synthesis
chitin. Because of its absence, the link between endo- and
exocuticle: it stratifies, and the insect can not complete
the process of pupation. Under the influence of MIS - various violations
molts throughout the development cycle from the egg to the beginning
pupation and the absence of pupae. The appearance of pupae is evidence
expiry of the ISX.

Under the influence of AUC: disturbances of morphogenesis throughout the entire development cycle, the absence of emigration (appearance), the appearance inferior imago or individuals with signs of the preceding phase development - "adultoids."

When assessing the activity of PPH, the main conditions are use in experiments strictly aligned by age (not less than 5-6 hours since the last molt) of the biological material: in evaluating the analogues of the juvenile hormone (AYG) use larvae of late age; when evaluating activity inhibitors of chitin synthesis (ISX) - larvae of the earliest ages for all biological objects.

The scales take the sample of the substance or to determine its activity, and a series of concentrations are prepared. In If the product is a liquid form, it The shaking is intensively stirred and then the sample is taken, if it is granules or powdery forms, then the sample is taken without preliminary manipulations.

Methods for assessing the activity of PPH.

Evaluation of activity PPH conduct on larvae yellow fever mosquito Ae. aegypti in the AYG test - 4 years of age; larvae of rat fleas X. cheopis, indoor flies M. domestica, red cockroaches B. germanica of late ages insect cultures; when estimating the MIR in experiments use Early age of larvae of these species.

Method for assessing the activity of PPH against mosquitoes.

When testing PPH against larvae of mosquitoes, they are vessels with water, which contain larvae of mosquitoes of the IV age at AYG test, and 1-2 ages when testing ISX. Observations for their development is carried out constantly, daily registering individuals with signs of the beginning of pupation, then - forming a pupa.

The indicators of AYG efficiency are:

1) absence of a normally formed pupa;

2) the presence of individuals who could not free themselves from the exuvium;

3) individuals who could not rise from the surface of the water.

Indicators of the effectiveness of ISX is the number of deaths larvae during the next molt. Accounting for the effectiveness of daily to register violations in their development during the 4-5 days. Count the number of larvae with morphogenetic violations.

When evaluating the effectiveness of PPH in the form of briquettes (pellets) proceed from the consumption rate: 1 briquette weighing up to 10 g per 10 m^2 of water surface. Strictly withstand the temperature required for development of larvae 27 ± 2 ° C, because depending on the temperature the terms of development are changing. The efficiency is monitored daily, registering the number of deformed larvae. Because of the slow

diffusion of DW into water from this form, in contrast to liquid and Powdered forms, when using which the DV immediately gets in the water, the effect manifests itself more slowly.

Method for assessing the activity of PPH against houseflies.

Preparations based on PPH are introduced into the substrate, where the development of preimaginal stages occurs in the period when the larvae of the first (ISX) or the last (AYG) age predominate. The indicator of efficiency is the absence of normally formed pupae (ISX) and the absence of a normal formed imago (AYG).

Method for assessing the activity of baits based on PPH in relation to house flies. Food bait with regulator of development of insects put in a cage size 30 × 30 × 30 cm, which is launched 50-100 imago 3-4-day indoor flies. In the same tank put substrate for oviposition (a mixture of bran with water and milk) and drinkers with water. Observations are conducted daily for 5 days prior to receipt oviposition.

Strictly withstand the temperature necessary for development larvae 20 ± 2 ° C, as the temperature varies with temperature development time: at 20 ° C - 4 days, at 16 ° C - 6-8 days. Accounting conducts daily.

Method for assessing the activity of PPH against rat fleas.

In the substrate for the development of larvae (a thin layer of sand,
in advance, to avoid molding,
2 hours and mixed with dry standard albumin and beer
yeast: 3 g of albumin per 10 g of sand), a solution of PPH in acetone is added.
After evaporation of the solvent, 30 flea larvae of the last age

is placed in a substrate. Efficiency is assessed by counting
daily larvae with metamorphosis.

Performance indicators:

1) disturbances in development of larvae;

2) the absence of a normally formed cocoon;

3) lack of viable imago.

Method for assessing the antigenic activity of antipyretic anthelmintics.

Activity of baits containing PPH against colonies
ants is estimated according to paragraph 2.3.2.

Method for assessing the activity of food bait with PPH against
cockroaches.

Food bait with PPH put in plastic boxes in size
20 × 20 × 30 cm, in which imago and larvae of cockroaches
(males: females: larvae in the ratio 1: 1: 4). In the same tank put
drinking bowls with water. Observations are conducted daily for 7 days,
counting the number of larvae with morphological changes.
Individuals with obvious disabilities in development appear no earlier than
in 5-7 days.

Accounting for the effectiveness of PPH:

1) the number of individuals with impaired (strongly melanized
larvae, intermediate forms with signs of the imago);

2) lack of viable ootec;

3) the generation of the normal generation.

The method of studying insecticidal fumigants for
based on PPH (hydroprene) against cockroaches.

To determine the biological activity of insecticidal

fumigiruyuschih devices (tools), made in the form of a disk-fumigator, in which AYG hydroprene is used as DV,

you need a special installation. This is due to the fact that the pairs hydroprene, evaporating from the surface of the fumigator disc (or other devices), have the ability to penetrate into cracks and cracks, where hiding cockroaches, and destructively affect them.

Experiments are carried out in a system of communicating with each other with using an adapter 15 × 15 × 30 mm box of transparent plexiglas 200 × 200 × 200 mm and a labyrinth with a lid of 200 × 200 × 40 mm.

This design allows you to create the length of the insect run in one labyrinth equal to two meters, keeping compactness installation, and to monitor the effect of hydroprene at the level of model group of cockroaches in conditions as close as possible to natural, for an extended period of life of insects.

Labyrinths serve as a refuge (imitation of cracks and cracks), and therefore they must be blacked out by a black opaque paper. In the experimental version, fumigators (or other similar devices), feeders and drinkers with water. In the control variant, the same conditions are created in the boxes, but without disks-fumigators. The experiments are set at room temperature air 20-24 ° C and natural light-dark mode.

The experiments are performed in 3 replicates. In each replication 60 cockroaches (10 females, 10 males and 40 larvae) are used with taking into account the natural ratio of individuals in the population of redheads cockroaches 1: 1: 4. Accounting for dead cockroaches, as well as monitoring for emergence of melanized individuals and cockroaches from morphological deviations are carried out on 1, 2, 3, 6-10, 13-14, 21, 28 day and further through 5, 6, 7, 8, 9, 10 weeks. In addition, they track the appearance of ootecs in females and the process of the emergence of larvae of the first age (within 10 weeks) and are added to the table. For determining

target effectiveness of the test substance with AAG is estimated:
absence of adults and the appearance of melanized and ugly individuals, and
also the absence of population growth. Statistical processing
results (the error value of the sample mean) are made according to
mathematical-statistical methods used in biological
research.

Performance indicators.

When PPH is introduced into the habitat - liquid forms:
deformed larvae-mosquitoes - 2 (ISX) or 4 (AUG) age,
after 2-4 days at least 90%, fleas - after 3 days at least 95%;
floating forms (briquettes, granules): deformed larvae
mosquitoes after 4-5 days at least 95%; pellets for fighting flies:
deformed fly larvae in 4-5 days, not less than 95%.

Food bait with PPH:

a) for flies: sterility of females, absence of oviposition through
5 days, viable eggs no more than 5%, deformed larvae
in 2-3 days not less than 95%;

b) for cockroaches: deformed larvae after 5-7 days are not
less than 95%.

Disks-fumigators with AYU for cockroaches: in 2 weeks
the appearance of individual melanized and deformed larvae.

2.7. Methods for assessing the activity of attractants (pheromones)

Attractants (pheromones) subdivided on sexual,
Aggregational, trace, anxiety and laying.

Sex pheromones are the main means of communication
individuals of arthropods of different sex, providing the process of pairing.
The smells that the female arthropods sing out are the main

way, to attract males. Pheromones can be excreted and
males for attracting and sexual arousal of females.

Aggregational pheromones predispose the existence of
groupings of insects in a certain place. Thus, for example,
Aggregational pheromone in cockroaches is active. Main quantity

This pheromone is found in the feces of cockroaches.

Trace pheromones - this chemical stimulus insects mark the ways of their movement; pheromones oviposition insects mark places that are optimal for oviposition; pheromones of anxiety are chemical stimuli by which insects inform each other about a possible danger.

In the composition of various formulations used in practice of medical pest control are used as sex pheromones, and aggregation pheromones to attract individuals to insecticide bait or sticky surface.

Estimation of the effectiveness of attractants and pheromones is carried out on imago domestic flies M. domestica and red cockroaches B. germanica.

Method for assessing the activity of attractants (pheromones) for redheads cockroaches.

Estimation of the effectiveness of attractants (pheromones) is carried out on red cockroaches of insect culture. For this, a box of plexiglass 20 × 20 × 30 cm, on the upper part of the inner surface which has a strip of Vaseline 2 cm wide, which prevents crawling of insects. In the box, 20 females, 20 males and 80 larvae of II-IV ages. Next, the attractant (pheromone) is placed on adhesive surface in the center of the box. At the same time, option: the box is placed on an adhesive surface without pheromone

similar area. Performance accounting is carried out after 24 hours. The repetition of the experiment is threefold.

Method for assessing the activity of attractants (pheromones) for indoor flies.

Two identical size 30 × 30 × 30 cm cages are prepared, into which put 50 pieces of adult indoor flies. In the experimental version, in put a sheet of paper 5 × 5 cm in size with the paper applied on it entomological glue and the test substance. In the control Sadok - a similar sheet of paper with only glue. Observations lead to for 3 minutes, count the number of adherent flies in the experimental

and control variants. Then calculate the coefficient

Attractive action (KAD) (experience - control): 50.

Method for assessing the activity of an attracting or intimidating

actions for houseflies.

Use an olfactometer consisting of two sealed chambers

volume of 0.5 m_3 , between which a gauze cage with two

cylindrical transitions to each chamber. Outlets

transitions opening into the chambers are arranged on the principle of vertices,

To prevent the flyback of flies from the experimental chamber into the tank.

In one chamber, a filter paper medium or circle is placed

(diameter 5-10 cm) with applied test substance or

drug, suspending it in the center in the lower third of the volume. In both

and put water bottles in the cage. Olfactometer

are arranged in such a way as to exclude the influence of phototaxis of flies

on the preference of the camera. Flies in quantities of about 500 pieces are produced

in the cage and register the number of individuals flying into the experimental and

control chamber. The experiment is carried out in duplicate, changing

The position of the camera with the tool (right or left). Accounting for flies

The number of people moving from the cage to the cells is counted every

30 minutes -1 hour for 6 hours. After 24 hours, the cameras are separated and

count the number of individuals in the chambers and in the cage.

Factor deterring action (RCD_{flies}) taking into account through

24 hours are calculated by the formula 20, where: 0 - the number of insects in

the experimental chamber, K - the number of insects in the control (idle)

variant, N - the number of insects in total in the experiment.

The coefficient of attracting (attractive) action (KAD_{fly})

when counted after 24 hours, calculate by the formula 21, where:

O - the number of insects in the test chamber, K - the number

insects in the control (idle) version, N - number

insects in total experience.

Method for assessing the activity of food baits containing

pheromone, for indoor flies.

Evaluation of the activity of food baits containing as

active substance, various insecticides in the composition with pheromone with cis-tricosene, is carried out according to the method outlined in item 2.3.3. with modifications. In a 30 × 30 × 30 centimeter cage is placed bait with a pheromone, in the second tank - the same bait without pheromone. Observations are conducted for several hours, conducting Accounting after 15 minutes, 30 minutes, 1, 2, 3, 6 and 24 hours. Count coefficient of attractive action (CAD), and mortality,%.

Performance indicators.

Attractants (pheromones) (sex, aggregation, food):

a) for flies in 3 minutes. KAD at least 50%,

b) for cockroaches in 24 hours, the KAD is at least 50%;

c) when tested in chambers when recorded in 24 hours - for flies Kadmuhi not less than 20%, the flyweight code - not less than 40%, when recorded through 24 hours.

2.8. Methods for determining the effectiveness of insecticidal, acaricidal and repellent means in full-scale conditions

2.8.1. General requirements for testing in field conditions

Based on the study in the laboratory of an insecticidal, acaricidal, growth-regulating, repellent activity of substances and their formulations determine the effective concentrations and norms of consumption in relation to different types of arthropods, having epidemiological and sanitary-hygienic value (spectrum of action) and the estimated multiplicity of treatments.

Since the real conditions for the use of funds significantly differ from laboratory ones, it is often important, and sometimes necessary to conduct tests in full-scale conditions. Under full-scale conditions real conditions for the use of disinfectants are understood. The objects in this case are living quarters, inhabited synanthropic insects, natural biotopes, transport means, etc.

At the first stage, optimal treatment regimes in
depending on the type of objects and the equipment used, possible
side effects that can occur in humans,
contact with the preparation (conducting processing,
in treated rooms, or using repellent
facilities). These tests are conducted on a limited basis
objects.

2.8.2. Study of the effectiveness of insecticidal agents of acute actions in full-scale conditions

Study of the effectiveness of insecticides in relation to
cockroaches, bed bugs and fleas. When examining an object on
The presence of bed bugs and cockroaches use visual
Control, noting the location of insects and their number.
The degree of occupancy of insects in the room is estimated by the following
indicators (see table). A visual assessment of the number of cockroaches in
object is advisable to spend during the hours of their activity - in the evening.

Accounting for the number of cockroaches can be carried out by placing traps
different types: cardboard shelters with sticky mass, as well as traps,
containing as bait attractants or pheromones of cockroaches
in combination with glue. Traps can be used
Glass jars with a capacity of 200-250 ml, in which as bait
use pieces of bread moistened with beer, brewer's yeast, pieces
apple. The traps are set at the rate of 1 specimen. for 10 m^2 of floor and not less than
2 copies. in rooms up to 20 m^2 .

Insects	Degree of occupancy of a room of 20 m^2		
	Small	Average	High
Bed bugs	Single insects	Up to 10 congestion sites	Over 10 places accumulations insects
Sinanthropic cockroaches	Single 3-5 copies		More than 5 copies

Insecticides of contact action are applied to the surface of walls
and objects of the environment in the habitats of insects. Consumption
liquid is 50-100 ml per 1 m^2 , dust - 2-5 g per 1 m^2 in

depending on the type of surface to be treated and the
substance in the dust.

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Accounting for the effectiveness of treatments is carried out for the first time in 24 hours after treatment, then every 5-7 days for a month. When
After the treatment, the number of
dead insects (by sex and age). Taking into account the effectiveness
the means activating the output must be used
Bugs and cockroaches from shelters (means in aerosol packages).
If no live insects are found within 2 weeks,
follow-up examinations are carried out every 2 weeks for 2
months.

When assessing the effectiveness of treatments with respect to fleas
use sticky sheets of 20 · 30 cm, which are laid out on the floor under
objects of the environment from the calculation of 2 sheets per 10 m^2 of area. If on
1 leaf during the first day was an average of no more than 2 fleas,
believe that there are "single fleas" in the room, from 3 to 10 - "fleas
a lot ", more than 10 -" a lot of fleas ".

Performance indicators.

Effective means are those that provide 100%
death of insects in objects within 1-2 days and possess
residual insecticidal action for at least 3 weeks. For
one-stage destruction of insects can be selected
acute agents in which residual insecticidal
activity does not exceed 3 days.

Study of the effectiveness of insecticides in relation to
flies. When studying the larvicidal activity of organic agents
Waste in collections of various types in which development takes place
preimaginalnih phases of flies, treated with aqueous solutions
(emulsions, suspensions) and dusts of insecticides. Consumption rate
working solutions (emulsions, suspensions) is 0.5-5 liters per 1 m^2 for

solid waste with a layer thickness of up to 30-50 cm, with a layer thickness substrate over 50 cm - 2-12 liters per 1 m^2. Consumption rate dust (dust) - 150-300 g per 1 m^2. At the same time, less than 3 objects of the same type.

Before processing, preimaginal phases of development of flies (larvae and pupae) in each a separate biotope. When counting the number of larvae and pupae of flies in solid waste accumulations in garbage containers, garbage piles, Composts in at least 5-6 points discard the top layer of garbage or manure on sites measuring approximately 20 · 25 cm and marking presence of larvae on a scale; there are no larvae (0), single larvae - up to 5 on the site (+), larvae occur in dozens (++), larvae are found in hundreds (+++). In clusters of liquid waste, The larvae are noted on the same scale when examining the surface of the substrate.

The effectiveness of insecticides is checked every 3 days in within 2 weeks after treatment. In accounting, dead larvae in the treated substrate, as well as the emergence of new clutch and development of larvae in the treated and new substrate. An effective means is considered to be a death of 98-100% larvae (pupae) for 5 days.

When studying the effectiveness of development regulators in 24 hours after treatment of the substrate, at least 3 samples are a 20 · 40 cm plot containing pupae. The samples are transferred to laboratory and kept at a temperature of 23-25 ° C for 10 days, counting the number of departed flies. Controls are samples, taken from an untreated substrate. Means are considered effective, if it ensures the death of 98% of pupae.

Contact activity of insecticides for flies determine when processing non-residential premises of various types, where there is a constant flight of flies: garbage chambers, utility rooms food facilities, toilet facilities, etc. Indoors

selectively irrigate the surface with suspensions (emulsions,
solutions) in concentrations selected at laboratory
experiments and ensuring complete loss of flies. In one
At least three objects are cultivated in the settlement.

Processing surfaces conduct of spraying
equipment, irrigating places of concentration of flies (walls near window
frames, doors, window panes, plafonds, etc.), but not more than 10%
surfaces of the walls of the room. The rate of liquid consumption for
absorbent surfaces is 50 ml per 1 m^2, for
absorbent - 100 ml per 1 m^2. The calculation is carried out by the number of DV
per 1 m^2.

A day before the treatment in the premises,
flies with the help of sheets (tapes) of sticky paper or sugar baits with
a highly active intestinal insecticide. Immediately before
Visually count the number of flies by counting
average (of three counts) the number of insects per 1 m^2 of surface
walls. The sticky sheets of 25 · 30 cm size are calculated from the calculation
1 sheet per 10 m^2 of floor.

The results of processing are taken into account according to the number of dead and live
flies in the premises in the morning hours 2 times a week for
3-4 weeks. After each count, the flies are removed from the premises.

An insecticide that provides death is considered effective
more than 95% of flies for 3 hours and retains residual insecticidal
action on the treated surfaces for 10 days.

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In order to establish intestinal activity, insecticides
studied in the form of liquid and dry baits. For the preparation of liquid
baits use 10% sugar syrup in which they dissolve
the necessary amount of insecticidal agent. Capacities,
containing 50-100 ml of bait (which is impregnated with cotton wool,
filter paper), placed on the windowsills, in closed
shop windows, etc. at the rate of 1 capacity per 10 m^2 floor area.
As they dry up, water is added to the previously noted level,
to maintain the necessary concentration of insecticide.

For the preparation of dry baits 40-60% solutions are used
sugar and the necessary amount of insecticide. The solution is applied by brushing
stripes 3-4 cm wide along the border of the window pane and to other
non-absorbent surfaces at the rate of 100 ml of solution per room
an area of 50 m^2. Granulated forms of food baits
spread on the substrates, which are placed in the places of greatest
fly flock according to the Instruction for practical tests
facilities.

The effectiveness of baits is taken into account in a day and a further 1 time
in 5-7 days during the month. If the bait retains its effectiveness
and further, counts are conducted once in 2 weeks for 3 months. When accounting
count the number of dead flies in a vessel with a bait and in a radius
50 cm from it and note the presence of flying flies in the room in
morning hours before the start of the flight of new insects. Additional 1 time
in 5 days, the effectiveness of the bait method with
using tapes (sheets) of adhesive paper, placed at the rate of 1 tape
(sheet) per room of 20 m^2

Performance indicators.

The liquid baits that provide
death of 95% of flies in the premises for 3 weeks, dry - death of 95%
flies in the premises for 2 months.

The study of the effectiveness of agents in aerosol and
non-propellant packages intended for destruction
flying insects.

Study of the insecticidal activity of agents in aerosol and
non-propellant packages in relation to indoor flies
spend in apartments, as well as in public facilities, not
in less than 10 rooms. The day before the treatment in
the number of flies using sheets (tapes)
sticky paper or sugar baits. Visual accounting of numbers
flies spend immediately before processing.

The product from the package is sprayed into the air with closed doors and

windows. The treatment of the premises starts from the opposite of the entrance end, gradually retreating to the front door. Remove the cap from the packaging and pressing the spray head, direct the aerosol jet to air and places of insects accumulation, moving the package along treated area at a distance of 50 cm from the surface. Time spraying means from the package depends on the content of the propellant, type of spray head and is indicated in the test facility. After 15 minutes. after processing the room air through air flow. Depending on the composition means, the time of processing the room and its ventilation can change.

The effectiveness of the agent is monitored visually in 15 minutes. after its application, then after 6 and 24 hours. When check the acute effect of the drug and possible residual

action - the time during which the death of flies occurs, flying into the treated room. Monitoring is carried out by inspecting the premises, noting the dead and paralyzed flies, and by polling the population.

Performance indicators.

The agent is considered effective if within 15 minutes. total insect death in the treated indoors.

The study of the effectiveness of agents in aerosol and non-propellant packages intended for destruction flightless insects.

Study of the insecticidal activity of agents in aerosol or non-flying packages for flying insects apartments and in public facilities, not less than in 10 rooms for each type of insect. One day before the event The object is examined in order to establish the degree of its population, using the methods and tools outlined higher.

The product is sprayed with open windows and windows. Withdraw from packing the cap, lightly press the spray head and

apply to sites where insect clusters are found or
places of their possible habitation. In this case, the packaging (balloon) is
distance of 20 cm from the surface, promoting it along the processed
sites. The rate of consumption of insecticide is 10-20 g per m^2
treated surface.

When treated against bed bugs irrigate places
detection bedbugs: beds, sofas, plinths, places of separation
wallpaper, cracks in the walls, the back of the paintings, carpets. Not allowed

hit the insecticide on polished surfaces. After
The end of the treatment is ventilated by a through flow
air for at least 30 minutes.

Control of the effectiveness of the drug is carried out immediately after
processing object, then in a day, one or two weeks, and when
necessity and in later terms after pest control. Wherein
establish the population of the object by insects, using the same
gradation, as in the preliminary registration of the population, the reaction
insects on the agent (whether the aerosol release is activated quickly
insects from shelters), the timing of the onset of paralysis and death
of insects.

Performance indicators.

Insecticide is considered effective if within 1 week after
no active insects were found in the room.

A study of the effectiveness of bait stations in relation to
ants in full-scale conditions.

In urban conditions, full-scale experiments are conducted in
The areas in which the ants were visually noted. Baiting
stations are installed in the places of greatest congestion (next to
ants' nest) or on the routes of regular intensive
movement of insects - ant "paths"). Accounting
moving foraging ants are conducted daily, noting
reduction and complete cessation of movement of insects.
Disappearance moving foraging ants serves
indirect evidence of the suppression of the colony and death of the female and

brood.

In conditions close to anthropogenic (greenhouses, greenhouses, greenhouses, concrete and stone paths in the garden, foundations

buildings), as well as in country houses (especially on verandas, terraces, in baths) you can often notice black garden ants and ants M. rubra, moving from an open nature. Baiting stations are installed in the same way - in the places of the largest congestion and movement of insects taking into account a specific location (between flower vases and containers, between bricks in foundation, in crevices between logs, under concrete slabs, etc.), Noting daily decrease in the number of ants foraging finally - the death of the colony.

A study of the effectiveness of gels and pastes against ants in actual conditions.

Natural experiments are carried out in the premises in which Ants were found. The gel is applied in the form of dashed lines (strokes, drops) along the skirting, in the cracks, cracks and other places possible habitat and movement of ants. It is possible to use a paste (gel) in the form of baits on a plastic substrate, which are set in In the vicinity of the cluster ants or intensive movement of ants ("paths" of working individuals).

Gels, containing insecticides acute actions (intended for a number of creeping insects) exert The pernicious effect on the foraging ants themselves. Gels, destined to destroy only the ants, allow workers of ants perform the function of foragers and transfer poisoned bait in a nest with a female and brood. As a result, exchange of food between ants die females, postponing eggs, and ultimately the whole family. Traffic intensity accounting ants are noted daily until it is completely stopped.

Study of the effectiveness of insecticides in relation to

Imago mosquitoes.

In the village choose the premises in which

concentrated malarial mosquitoes of the genus Anopheles (cribs, sheds,

living rooms, etc.). Before processing,

mosquitoes.

Sites premises, which are by the mosquitoes

The medium is treated with working solutions at a rate of 100 ml per

1 m^2 . In one locality, each preparation is not treated with

less than 3 rooms in concentrations selected at laboratory

tests.

The effectiveness of the insecticide is determined either visually,

counting the next day after treatment and then every

3-5 days the number of dead mosquitoes in the premises, or with the help of

transparent plastic cones attached to the treated

To surfaces with adhesive tapes In each cone with

The exhauster is placed 10-20 mosquitoes for 30 minutes. After this, mosquitoes from

Cones are transferred to a clean cage and left for a day. In the cages

place cotton wool soaked in water. Such tests are carried out first

once a day after treatment and then once every 7-10 days until the time,

As the death of mosquitoes will drop to 70%.

Control rooms are those not processed

insecticide. In these rooms also put a cone, because

It is necessary to take into account the trauma of mosquitoes when planting them in a cone and

transplantation into cages by an exhauster (comparison of death in experiment and control).

Performance indicators.

Effective consider facilities (their concentration),

the death of 100% of mosquitoes during the first 3-4 weeks

after treatment and not less than 95% during subsequent periods

observation.

For the treatment of individual foci (according to indications), there may be

used funds that do not have a long residual effect,
providing 100% death of mosquitoes within 5-7 days after
processing.

When studying the degree of irritability of mosquitoes to the studied
100-200 mosquitoes are released into the treated premise and
for an hour they observe their behavior. If mosquitoes sit on
treated surfaces and die without showing signs
There is no irritability, and the insecticide may be
recommended for use. If mosquitoes are restless and try
fly out of the treated room, then there is irritability, and
studied insecticide in the practice of antimalarial treatments
can only be recommended after a second test and
confirmation of the absence of irritability of mosquitoes.

When studying the effectiveness of insecticides in relation to
Imago mosquitoes of the genus Culex are treated with 2-3 cellars with a high
the number of mosquitoes (more than 100 specimens / m_2)
selected in laboratory tests and the rate of application
100 ml / m_2. After a day, mosquito numbers are counted, and
determine the effect of insecticide on the walls of the cellar with the help of
cones according to the method described above. Mosquitoes from cones are placed in
their deaths are recorded after 24 hours. The following accounts
the number of mosquitoes and experiments with cones take place every 7-10 days
up to when the drug will cause death of less than 70%
mosquitoes. Insecticides that provide 100%
death of mosquitoes within the first two weeks after treatment and not

less than 90% of death in the following month. In separate objects
(according to indications) insecticides with a short
residual action on the treated surfaces (5-7 days).

Study of the effectiveness of insecticides against larvae
mosquitoes.

The agents are tested in at least three concentrations in
water bodies with an area of at least 1-5 m_2. To determine the effectiveness
At least three water bodies of the same type are selected for one concentration.

Working solutions or powders from spraying or
spraying equipment processing the surface of the reservoir from
calculating 100 ml (g) per 1 m^2. The granules are evenly spread by hand
at the rate of 20-30 g per 1 m^2. The rates of consumption of insecticide are calculated in
grams DW per 1 m^2, if the depth of the reservoir does not exceed 20 cm; if
the depth of the reservoir is greater, then the calculation is made for the volume of water in grams
DV for 1 m^3.

The efficiency of the treatment is assessed by comparison
the number of mosquito larvae before and after treatment after 1, 2, 5, 7 and 14
days in the treated and control reservoirs. Evaluation of the
the results are carried out according to the formula 3.

The number of larvae of different ages is taken into account
with a standard net (diameter - 20 cm, depth - 25 cm) or cuvette with
counting the number of larvae of each age by 1 m2. Samples do not take
less than 10 places evenly over the entire area of the reservoir. During the period
tests in water bodies record the temperature, water pH, degree
overgrowing pond vegetation, power flow,
contamination of water with organic substances.

The residual action of the agents is determined in mosquito larvae
insect culture. For this, from the treated pond,

in 0.5 liter of a container of 250 ml of water, into which 25 larvae are placed
II-III age. The dead larvae are counted after 1 and 2 days.
Every 5-7 days, the experiment is repeated until the number of larvae,
of those killed in water taken from the treated reservoir will be below 70%.
Simultaneously put the control. A remedy is considered effective if
Within 1-2 days it ensures 100% death of larvae in the reservoir.

The study of the effectiveness of insecticides in a special
aerosol packaging intended for the treatment of aspen nests in
actual conditions.

A special aerosol package is provided with a head that
provides an aerosol spray with a length of up to 5 m.

The following conditions and measures must be observed:
precautions for the treatment of aspen sockets: Not recommended

to process one test. To process the slots
cool days are favorable, as wasps are more active and more aggressive
in warm weather. The most suitable time for processing nests -
early morning (before sunrise), when the activity of wasps is the smallest,
or after sunset at dusk, when almost all working individuals
are in the nest. This requirement is also consistent with the environmental
regulations - conduct insecticide treatments early in the morning and
Late in the evening to avoid possible contact of bees with poisons.
The specified time of day is also a factor limiting
possible unwanted contact of wasps with humans and animals.

Do not use a flashlight or
Another light source, since this can provoke activity
wasps. Conducting treatment should wear protective clothing, good
covering the head and neck, hands (cloak or jacket of
water-repellent fabric with a hood, tight gloves). Not

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Before processing, use perfume and use
alcoholic beverages, as the wasps react aggressively to the sharp odors.

The testers find an aspen nest that is located in
their reach. One of the testers processes the socket,
not removing it from the attachment point, from a distance of at least 1.5 m in
for 2-4 seconds, which corresponds to a rate of flow of 40 g / m_2 . Then the second
the testator quickly puts on the jack a sealed transparent
a polyethylene bag and tightly knots it. Accounting for efficiency
treatment is carried out after 1 hour and 12 hours after the appearance of live or dead
wasps in a bag.

The study of the effectiveness of special attractive and
Attractive insecticide baits designed to capture and
destruction of wasps, in full-scale conditions.

Lures designed to capture and destroy wasps
are divided into two types:

a) attractive bait based on carbohydrates without insecticide -
such baits are placed in traps (for example, bottles with water),
of which the insects attracted by the attractant can not escape
outside; Several traps with bait are placed indoors or

outside the structure, and take into account the trapped wasps;

b) bait based on protein feed, containing an insecticide
delayed action (for example, avermectins) or a regulator
development of insects - poisoned protein food insects are
nest and fed to the larvae; spread of poisoned food
occurs inside the nest via trophallaxis.

Performance indicators:

a) insecticides in a special aerosol package,
intended for the treatment of aspen nests: the death of insects through
1 hour and 12 hours is not less than 100%;

b) baits based on hydrocarbons with attractant,
trapped: catchability after 2 days at least 10 individuals;

c) bait based on protein feed containing an insecticide:
the death of the colony in 3-4 weeks.

2.8.3. Study of the effectiveness of insect development regulators in actual conditions

The agents are tested in at least three concentrations on
water bodies with an area of water surface from 1 to 5 m^2. For determining
the effectiveness of one concentration is selected by at least three water bodies
one type.

Working solutions or powders from spraying or
spraying equipment processing the surface of the reservoir from
calculating 100 ml (g) per 1 m^2. The granules are evenly spread by hand
at the rate of 20-30 g per 1 m^2. The norms for the consumption of PPH are calculated in grams
DV for 1 m^2, if the depth of the reservoir does not exceed 20 cm; if the depth
the water is larger, the calculation is carried out for the volume of water in grams of DV
for 1 m3.

The efficiency of the treatment is assessed daily by comparison
number and appearance of mosquito larvae before and after treatment to
Obvious effects of molting in treated

water reservoirs.

The number of larvae of different ages is taken into account with a standard net (diameter - 20 cm, depth - 25 cm) or cuvette with counting the number of larvae of each age by 1 m2. Samples do not take

in less than 10 places evenly over the entire area of the reservoir. During the period tests in water bodies record the temperature, water pH, degree overgrowing pond vegetation, power flow, contamination of water with organic substances.

The residual effect of PPH is determined on mosquito larvae insect culture. For this, a treated pond is 0.5 liter of a container of 250 ml of water, into which 25 larvae are placed 1-2 age in the case of MIH and 4 years in the AYG test. Accounting dead larvae spend daily. Every 5-7 days experience repeated until the number of larvae perished in water taken from the treated pond, will be below 70%. Simultaneously put control.

Performance indicators.

Means are considered effective if within 25-30 days they are provide violations of molting or death of pupae in water taken from the treated pond.

2.8.4. The study of the effectiveness of acaricidal (insectoacaricidal) resources intended for the processing of natural habitats for the purpose of destruction of ixodid ticks

The study of acaricidal activity of agents is carried out on The territory where a high number of mites is marked (no less than 20 specimens. for 1 km of the route with the standard registration of the number of ticks flag) in period of rise or peak of numbers. For taiga mites this the period falls on the middle of April - the first half of June. AT The drop in the number of mites tested by acaricides is not spend.

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Study effectiveness of concentrates emulsions and wettable powders. To test the drugs on the selected

Territory is divided into square plots measuring 10 · 10 m,
an area of 0.01 ha. Three plots must be control and
3 plots per each tested rate of expenditure of the facility.

The perimeter of each plot is marked with a neutral strip
with a width of 1 m. Together with a neutral strip, the size of each plot

equals 12 · 12 m, the area is about 0,0144 hectares. In order to increase
The number of mites in the plot at the center of each of them is
30 ticks (15 females and 15 males) collected on the flag in the territory, not
treated with acaricides. Experience begins with the following
day after marking the sites and replanting ticks.

Process acaricide every plot, including
neutral strip. The control plot is arranged so that
To prevent insecticides from entering it (drift by wind during
treatment, drainage of water in the rain). Processing plots are carried out
insecticide at the concentrations recommended in
project Instructions for use. For processing use any
sprayers, providing shallow-drop atomization.
The treatment is carried out so as to ensure a uniform coating
the tested preparation of the entire plot area.

Ticks are counted at the same time on control and experimental
before treatment, 3 days after treatment, and then
weekly for 1-2 months until the ticks disappear completely.
control plot. The records are held in the morning after the departure
dew. If the weather is overcast, without a noticeable increase in midday
temperatures, accounting can be carried out in the afternoon. For accounting on experienced
plots and in control use different flags that must be

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are respectively marked. At each control plot,
At least 10 mites must be registered in the first record.

The absence of ticks or fewer ticks on it indicates
the poor quality of the experiments and should be considered
the question of the repetition of experience in another territory. On each plot
the accounting is carried out by shuttle travel so that the total length of the route
was not less than 0.1 km, preferably 0.2 km (10-20 parallel
moves on the site with a side of 10 m). Evaluation of the results
is carried out according to formula 8. Calculation of the effectiveness of the action of the acaricide
spend for each period of the control survey. Change
Seasonal performance indicators serve as a criterion for the duration
effect of treatments.

Performance indicators: the effectiveness of funds for 3 days -
not less than 95%, the duration of such action should be at least 30
days.

2.8.5. The study of the effectiveness of insecticacaricides,
designed to protect people from attacks by mosquitoes, midges and
other flying insects

Evaluation of the effectiveness of insecticaricidal agents is performed in
Natural conditions for mosquitoes of natural populations.

Prior to the experiments, three testers take the intensity
mosquito attacks by the method of A.V. Gutsevich "on himself" (accounting
unit - the number of mosquito plantings on the exposed forearm
tester for 20 minutes). For these experiments, a high
(from 80 to 120 plantings in 20 minutes on the exposed forearm of the test)
number of attacking mosquitoes. With such a number, consider
it is necessary to plant mosquitoes in 5 minutes, and then recalculate the received

data for 20 min. It is forbidden to perform these experiments in natural
foci of vector-borne infections, the causative agents of which are transferred
mosquitoes.

Preparatory stage.

Of pure white cotton coarse calico
3 control tests with a size of 70.0 · 30.0 cm.
should be in contact with experienced tests or hands contaminated

insectoacaricides! To prepare test tests on the wall in
A well-ventilated room is secured with a clean white

cotton coarse calico size 70 · 70 cm studied in

the aerosol form is evenly treated with this tissue, at the rate of application

the studied agent recommended for its use

(as a rule for aerosol and non-propellant aerosol

packages - 20 g / m2 of surface).

Evaluation of the effectiveness of the tool is carried out by 3 testers dressed in

Clean clothes from mosquito-bitten tissue. Testers' faces

must be protected by nets on the front of the hoods.

The testers are placed in a natural biotope (each test

bare only forearms, hands).

Control tests cover the entire surface of the brush and

forearm of each tester. To fix the tissue use

stationery rubber rings. The dough fabric should be free from tension

Adhere to the forearm, on the hand is allowed to stretch the fabric.

The number of mosquito bites is recorded through the control tissue

tests for 10 minutes. After the experience with the control tests similarly

experiments with experimental tests, registering the number of

mosquito bites through the tissue for 10 min.

Coefficient of protective action (CPD mosquitoes in percentages)

is calculated from the formula 19.

Each experiment is conducted on three tests by different testers

(repetition). Calculate the average performance score

means (CPD mosquitoes) for mosquitoes and its statistical error. Between

The tests are suspended in the laboratory at room temperature

22 ± 2ºC, relative humidity of 50-70% and natural light.

The tests begin after 1 day after the tests

and repeated to establish the duration of the protective action through

7, 14 or more days before the decrease in the CPR indicator of mosquitoes below

normative. The test frequency is determined by the test

laboratory (center). Duration of insecticidal action on the tissue

indicate when describing the properties of this tool.

Performance indicator: CPD mosquitoes - not less than 95%.

2.8.6. Study of the protective properties of tissues containing insectoctacaricide

Study protective properties fabrics, containing insecticacaricide, against mosquitoes, midges and other flying Insects also carry out in natural conditions similarly as described above. From the studied tissue area of 2 m^2 cut out from different places 3 test tests of the same size as the control tests.

Performance indicator: CPD mosquitoes - not less than 95%.

2.8.7. A study of the effectiveness of repellents in relation to bloodsucking insects in full-scale conditions

The study of the protective effect of repellents in relation to flying sucking insects intended for application on the skin (creams, lotions, emulsions, napkins, pencils, aerosols). Tests of the effectiveness of repellents from flying bloodsucking insects spend in natural biotopes with high and average number of different attackers per person bloodsuckers to determine the spectrum of protective action. Repellent the agent is applied to the entire surface of the exposed forearm or tibia tester in the rate of consumption of 0.1 ml (g) per 100 cm^2 of skin. The forearm of the second arm (the shin of the second leg) is treated in the same the rate of consumption by the standard of the proposed efficiency category. As a reference, DETA is used in the concentrations indicated in 2.5.1. The COD is recorded after 15 minutes. after application.

The criterion for the end of the protective action is registration 3 bites of insects in the forearm or tibia of one tester for 3 minutes. A test tester during the tests takes into account and

captures blood-sucking insects, produces all records, measures temperature and humidity, direction and strength of the wind, notes weather conditions, etc.

The test of each agent is carried out at least 10 times with various conditions (different biotopes, weather conditions, time of day). Based on the obtained data, calculate the CODE and the DDD value in relation to various groups of blood-sucking insects (mosquitoes, midges, slippers), which is expressed in hours. As additional tests of protective properties repellent means with different physical load of the testers.

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Performance indicators.

RCD - 100%, duration of repellent action in relation to mosquitoes in hours in accordance with the categories of effectiveness (see 2.5.1.).

The study of protective action against blood-sucking insects and mites repellents in the form of concentrates emulsions, aerosol and non-propellant packages, intended for application to clothing.

The processing of clothing (suits, head nets, etc.) is carried out in according to the recommended method of application (soaking in emulsion, spraying of the emulsion, spraying from the aerosol packaging). Tests are carried out by specialists dressed in processed clothing in places with a high number of nematodes and ticks. Untreated Clothing should not protect against insect bites. Efficiency protective action impregnated with repellent clothes are determined by ratio of the number of bloodsuckers attacking a unit of accounting time (20 min.) per test in the treated and control (untreated) clothing. An acute deterrent effect determines the day after treatment. In the future, one every 3-5 days until the loss of clothing protective properties. The duration of the protective action of the repellent is a period of time in a day until the protective properties remain at least than by 90%.

Performance indicators.

RCD - 100%, duration of repellent action in relation to mosquitoes in hours in accordance with the categories of effectiveness (see 2.5.1.).

2.8.8. Methods for studying the effectiveness of repellents in of ants in full-scale conditions

Evaluation of repellent activity is carried out on ants of genus Formica in full-scale conditions during the period of their activity (spring-autumn). Each facility is tested by at least three testers not less than in three replicates (total of 9 replicates).

The method of studying the effectiveness of repellent means, intended for protection from ants, when applied to clothing.

From the cotton coarse calico make test pants an area of 0.5 m^2 and treated with a means of 20 g / m^2 .

When tested, the processed leg is worn on the leg tester, tucking into the sock to avoid getting the ants under clothes and a foot in the shoes put on the place near the anthill, where an accumulation of insects. Ants come on shoes, after which rise up on the toe to the processed leg and crawl along her up. Record the number of ants crawling the line, treated with insect repellent, 50 cm long.

Calculate the RCD by the formula 12, which is equal to the fraction of ants (in percent), not crawled the treated area from the number of ants in experience. The test of repellent activity is carried out for the first time after complete drying of the fabric (about 1 hour after treatment) and then after 1, 2, 3, 5 days and more. To determine the duration repellent effect of the test is repeated until the CODE not less than 70% is retained. In between tests trouser legs suspended at laboratory at room temperature 22 ± 2ºC, relative humidity of 60-70% and natural illumination.

The method of studying the effectiveness of repellent means, intended for protection against ants, when applied to the skin.

Only substances having confirmation of the safety of their use on the skin of people. Learned The agent is applied to the bare shank of the test person at the rate of flow 0.1 ml (g) per 100 cm 2 of the skin surface. Testers should avoid getting ants under the clothes: put on socks with a dense rubber band roll up the pants so that they fit snugly to the leg above the knee. The foot in the shoes is put on the place next to the anthill where the congestion of insects. Ants go to the shoes, after which climb up on the toe to the treated shank and crawl along it up. Record the number of ants crawling the line, treated with a repellent (from the toe to the knee). Tests begin immediately after the application of the product. With the aim of Determination of the duration of repellent action (DRD) is carried out repeat the experiment every 30 minutes.

Calculate the RCD by the formula 12, which is equal to the fraction of ants (in percent), not crawled the treated area from the number of ants in experience. To determine the duration of repellent action the tests are repeated until the CODE is kept at least 70%.

2.8.9. The study of the protective properties of special clothing for protection from bloodsucking insects and mites

The tests are carried out in accordance with GOST R 12.4.296-2013 SSBT. Special clothing for protection against harmful biological factors (insects and arachnids) General technical requirements. Test methods.

2.8.10. Practical tests of insecticidal, acaricidal and repellent means

Practical tests - tests of disinfectants,
ongoing forces sanitary and epidemiological and
disinfection services at conventional disinfection facilities
activities using the available services
equipment.

Practical testing requires permission
health authority responsible for the epidemiological
situation in the country. Currently, this permission gives
The Federal Service for Supervision of Consumer Rights Protection and
well-being of a person.

Practical tests are carried out by practical
organizations in different regions, differing in climatic,
geographical conditions, species composition and seasonal dynamics
number of arthropods, etc. In carrying out these tests
determine the effectiveness of the tool in relation to the dominant
species of arthropods depending on the climatic conditions of the region
and the level of sensitivity of natural populations of arthropods to
insecticide, determine the duration of the residual effect of the agent
when used in different climatic zones, suitability for
standard equipment, the possibility of
recommend it for use by the public

Practical tests are carried out in accordance with
approved by the Federal Service for Supervision of Protection of Rights
consumers and human well-being.
practical tests and instructions for their conduct,
developed for each test means by specialists

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(entomologists, biologists, parasitologists, doctors, hygienists). AT
The instruction should include general information about the facility (composition
completely, the hazard class, for what purpose it is intended); technique
processing; Working concentrations and drug consumption rates for
relative to each group of arthropods for which the agent
is intended; method of accounting processing efficiency; measures
precautions for handling the product and first aid measures for

accidental poisoning.

The Instruction is accompanied by the forms of the necessary documentation (acts, questionnaires, questionnaires, etc.), which are filled by specialists practical organizations, directly supervising tests of the drug. In questionnaires, in addition to the effectiveness of the tool, note the presence or absence of side effects in humans, carried out treatments that used the tool or were in the processed objects.

Generalization and analysis of materials of practical tests The organization responsible for the preparation and conduct of such tests.

Prepared reports on the results of practical tests With the application of originals of the Acts of organizations that conducted tests are submitted to the Federal Service for Supervision of protection of consumer rights and human well-being

Precautions for working with natural populations arthropods. Many arthropods are vectors causative agents of natural focal diseases (malaria and plague, viral encephalitis and yellow fever, trypanosomiasis and rickettsiosis, tularemia, etc.). In connection with this, when collecting and following

work with natural populations of arthropods observe safety precautions!

When working in natural biotopes located in natural foci of vector-borne infections, staff should be instructed about the necessary precautions in attitude of arthropods. It is imperative that appropriate vaccinations and the availability of the necessary means of emergency prevention. When working with arthropods it is necessary to work in rubber gloves and gowns, exhausers should be equipped with pears or cotton filters.

When working with ixodic mites, the desktop should be covered with white paper and there should not be any extra items on it.

The researcher should not simultaneously monitor
several mites or allow the mite to disappear from the species.
People working with mites that carry the virus
Tick-borne encephalitis should be vaccinated against this infection.

Arthropods, survivors after the
experiment, destroy (brew with boiling water or freeze)
or retained for further research in accordance with
requirements. When it is necessary to collect collections, arthropods
is placed in 96% ethyl alcohol).

2.9. Apps

Application (recommended)

Indicators of the effectiveness of means of pest control

No. p / p	Product type	Investigated indicators	Standards
1.	Adhesive traps without insecticides in the form of houses for fighting cockroaches	Average catchability,%	
		for 7 days	not less than 90
		on the 14th day	not less than 95
		Trap resource	1 ginger cockroach / cm 2
		Validity, day	not less than 15
2.	Adhesive traps in the form of tapes, sheets, sticks to combat flying insects (flies, moths)	Average catchability for 2 days,%	not less than 95
		Trap resource	1 room fly / cm 2
		Validity, day	not less than 15
3.	Adhesive traps for catching fires (food moths) and clothes moths	Average catchability for 2 days, %	not less than 70
	Glue for catching insects	At a flow rate of 1.50 g / dm 2	
		Average catchability for 2 days, %	not less than 95
		Resource of glue	1 room fly / cm 2
	Insecticides		
	Insecticidal food bait to fight against cockroaches and ants (in		

the form of containers, tablets, balls, briquettes, pellets, Powder, pastes, gels, foams and etc.):		
a) DV - FOS, carbamates, pyrethroids, phenylpyrazoles (fipronil);	Acute action: Death,% for 2 days	not less than 70 *
b) DV - imidacloprid (gel)	Acute action: Death on the 3rd day,%	not less than 70
c) DV - hydramethylnon or other amino hydrazone, sulfamurides, neonicotinoids (thiamethoxam, acetamiprid), avermectins;	Acute action: Death on the 5th day,%	not less than 70
d) DV - boric acid, borax (gel)	Acute action: Death on the 5th day,%	not less than 70
e) DV - boric acid, borax (liquid bait)	Acute action: Death on the 5th day,%	not less than 70
Insecticidal food		

bait to fight against synatropic flies (liquid, dry sugar, granulated, plates, gels, etc.)		
a) DV - FOS (except chlorophos), pyrethroids, neonicotinoids;	Acute action: Death of flies after 24 hours,%	100
b) DV - chlorophos	Acute action: Death of flies after 24 hours,%	not less than 80
Insecticides in aerosol packages from propellants: to combat flying insects (flies, mosquitoes, etc.)	Acute action on flies: C15, mg / m3	not more than 15
	Q15, mg / m3	not more than 1000
	KT $_{50}$, min	not more than 10
Insecticides in aerosol and non-propellant packages to combat non-flying insects (cockroaches, bugs, ants, fleas)	Acute action for cockroaches: Death of cockroaches,%	
	in 10 minutes	not less than 30
	in 24 hours	100
	Residual action: death cockroaches after 24 hours at for a 3 day supply,%	Not less than 50
Insecticidal pyrotechnic facilities (drafts, tablets, cords): a) to combat flying insects	Acute action: death (%) after full fogging through 2 hours	100
b) to combat non-flying insects	Acute action when accounting through 24 hours:	
	Death of cockroaches,%	not less than 90
	Death of fleas,%	100
c) candles to combat mosquitoes	Death of mosquitoes (%) at 6 min burning 15 minutes after candle suppression	not less than 90
Insecticidal spirals for struggle from flying insects	Acute action: KT $_{50}$ for mosquitoes, min.	Not more than 7
	KT $_{50}$ for flies, min	not more than 60
Insecticidal dusts for struggle from flightless Arthropods and processing places of planting and breeding of flies	Acute action: The death of cockroaches, flies after 24 hours,%	100
	Residual action:	

	death of cockroaches after 24 hours when replanted for 3 days,%	100
Insecticidal pencils, Crayons, bars to combat flightless synanthropic arthropods	Acute action: Death of cockroaches after 24 hours,%	100
	Residual action: death of cockroaches after 24 hours when replanted for 3 days,%	100
Insecticide concentrates emulsions, microencapsulated,	Acute action: The death of arthropods through 24 or 72 hours,%	100

wetting, or water-soluble, or dispersible powders and tablets, suspoemulsions, flow for combating synanthropic arthropods	Residual action: death of cockroaches after 24 hours when replanted for 3 days,%	100
Means to combat larvae of mosquitoes:		not more given in
a) microbiological	SK 50 , mg / l,%	TU
b) FOS, pyrethroids, etc.	Larvae mortality after 24 hours,%	100
Facilities electrofusing for struggle from flying insects:		
for mosquitoes and other insects (except flies)	KT 50 for mosquitoes, min	
a) with mats (plates)	plates	not more than 7
b) liquid	fluids	not more than 4-5
c) tablets	pills	not more than 10
	Reserve mat (vial, tablets) for mosquitoes, hour	not less specified in NTD
For flies: on empenthrin (vaportrin) and Transfluthrin:	KT 50 for flies, min	not more than 9-12
on other volatile pyrethroids		not more than 20
	Reserve of means for flies, hour	not less specified in NTD
Fumigating devices for outdoor use air lantern with a candle and insecticide plate)	KT 50 , min	not more than 2
	Fund Reserve	not less specified in NTD
Fumigating devices for outdoor use air with a fensystem on batteries (pappelator)	KT 50 , min	not more than 2
	Fund Reserve	not less specified in NTD
Insecticides based on insect development regulators (PPH)		
When entering the habitat on basis analogues Juvenile Hormone (AGU)	Deformed larvae:	
- liquid forms	mosquitoes 2 (ISX) or 4 (AUG) age in 2-4 days,%	not less than 90
	larvae of fleas in 3 days,%	not less than 95
- floating forms (briquettes, granules)	mosquitoes 2 (ISX) or 4 (AUG) age of	
- pellets for combating flies	in 4-5 days,%	not less than 95
	larvae of flies after 4-5 days,%	not less than 95

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Food bait:	Sterility of females	Absence of oviposition
- for flies with AYG and ISX		in 5 days
	Viable eggs,%	not more than 5
	Deformed larvae through 2-3 days,%	not less than 95
- for cockroaches with AYG and ISX	Deformed larvae,% in 5-7 days	not less than 95
Disks-fumigators with AYUG	Melanized	and Appearance
- for cockroaches	deformed larvae through 2 weeks	single individuals
Pediculicidal agents in different forms for the fight with head, pubic, and pediculosis		
Lotions, concentrates emulsions, shampoos, soaps, etc.	Death of the imagos after 24 hours,%	100
	Death of eggs after 21 days,%	10-100
washing facilities, dust, suspension, creams, gels, emulsion, etc.		
Means for impregnation for warning infection with his lice	The loss of imago and larvae at contact60 min. when recorded through 24 hours, %	100
	The defeat of adults and larvae at contact for 180 min	100
Means of fighting moth and kozheedami		
Contact action	Acute action: Death of caterpillars of moths or larvae kozheedov through 72 hours,%	100
Fumigation action	Acute action: Death of an adult in a volume of up to 100 liters in 48 hours,%	100
	Knockdown flies in a volume of 10 liters in for 3-6 hours not less than,%	95
	The death of flies after 24 hours, not less,%	80
Repellent action	COD in olfactometer after 24 hours,%	not less than 75
Means of combating wasps		
Insecticides in aerosol packages for spraying into the air	Acute action on os (flies - test-an object)	
	With $_{15}$ mg / m $_3$	Not more than 10
	Q $_{15}$ mg / m $_3$	Not more than 1000
	KT $_{50}$, min	Not more than 10
Insecticidal facilities different forms for processing nests	An acute effect on cerebri, cockroaches - test object) with replanting on absorbent surface (15 minutes after processing):	
	Death,%	not less than 100
	Residual effect for 3 days, %	not less than 50

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Insecticidal food baits	Acute action: Death of flies - test object: Death in 24 hours, %	not less than 80
	Residual action: Death after 3 days,%	not less than 80
Bezinsecticidal traps with attractants	catchability after 2 days, individuals	not less than 10
Acaricidal and insecticacaricidal agents		
For of protection of people from ticks, fleas and mosquitoes (application on clothes and on fabric products)	Acute action: For ticks	
	KT $_{wed}$, minutes	not more than 5
	MB $_{cp}$, cm	not more than 50
	ISP	not more than 1,1
	For fleas	
	KB $_{5}$, copies.	not more than 3
	MB $_{cp}$, cm	not more than 20
	For mosquitoes	
	CPD $_{mosquitoes}$,%	not less than 95
To combat the gamma and scabious ticks at premises	Acute action: Death of ticks in 24 hours,%	100
	Residual action: Death of mites when replanting on 3 day on non-absorbent surface after 24 hours,%	not less than 80
To combat ticks indoor dust in the rooms	Acute action: Death of mites	
	in 15 minutes,%	not less than 85
	in 24 hours,%	100
For fighting with ixodial ticks at natural biotopes	Acute action: Efficacy for 3 days,%	not less than 95
	Duration of action, day	not less than 30
Tissues containing insecticacaricides		
For of protection of people from ticks, fleas and mosquitoes	For ticks	
	KT $_{wed}$, minutes	not more than 5
	MB $_{cp}$, cm	not more than 50
	ISP	not more than 1,1
	For fleas	
	KB $_{5}$, copies.	not more than 3
	MB $_{cp}$, cm	not more than 20
	For mosquitoes	
	CPD $_{mosquitoes}$,%	not less than 95
Insectorodenticidal means		
Baits insectic-indenticidal for simultaneous destruction fleas or mites and rodents	Acute action: replanting on mice feeding on bait in for 3 days, and counting through 24 hours of fleas or rat mites nourished individuals, mortality rate,%	Not less than 80
Repellent means		
For application on the skin	Acute action: CODE for mosquitoes,%	100

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	COD for ants,%	At least 95
	Duration of action:	
	ДРД для комаров, часы (по categories of effectiveness):	
	highest category	4 and more
	1 category	3 and more up to 4
	2 category	2 and more up to 3
	3 category	1 or more to 2
	4 category	not less than 1 (at low

		number of mosquitoes)
For application to clothing and fabric products	Acute action: CODE, %	
	for mosquitoes	100
	for fleas	95 and more
	for ixodid ticks	95 and more
	for ants	95 and more
	Duration of action: ДРД, day (by category effectiveness):	
	for mosquitoes:	
	highest category	20 and more
	1 category	10 and more before 20
	2 category	5 and more to 10
	3 category	3 or more to 5
	for fleas:	
	highest category	5 and more
	1 category	3 or more to 5
	2 category	2 and more up to 3
	for ixodid ticks	3 and more
	for ants	3 and more
Repellent bracelets, stickers and other products, containing repellents and intended for protection people from mosquito attacks	CPD,%	not less than 30
Acaricidally-repellent agents		
For protection against ixodid ticks and flying bloodsucking insects (application to clothing and articles of fabric)	For ticks	
	KT $_{Wed}$, minutes	not more than 5
	MB $_{cp}$, cm	not more than 50
	ISP	not more than 1,1
	For fleas	
	KB $_{5}$, copies.	not more than 3
	MB $_{cp}$, cm	not more than 20
	For mosquitoes:	
	Acute action: RCD,%	100
	Duration of action: DRD, day (by categories	

	effectiveness):	
	highest category	20 and more
	1 category	10 and more before 20
	2 category	5 and more to 10
	3 category	3 or more to 5
Attractants, pheromones		
For flies	KAD in cages for 3 min,%	not less than 50
	KAD $_{flies}$ in the chambers, for 24 hours,%	not less than 20
For cockroaches	Ring Road for 24 hours,%	not less than 50
Clothing to protect people from arthropods		
For of protection of people from ticks, fleas and nasal (flying bloodsucking insects)	For ticks	
	CCD $_{mites}$,%	not less than 98
	For fleas	
	CPD of $_{fleas}$,%	not less than 98
	For the louse	
	CCD of the $_{nasal}$,%	not less than 90
	Duration protective	

actions:	
a) clothes with permanent protective properties, years	2 and more
b) clothes from temporary protective properties, day	not less than 14

3. Rodentological research methods and evaluation criteria the effectiveness of deratization products

3.1. General provisions. Requirements for experimental animals and conditions of their maintenance

Deratization means are chemical and physical means used for killing and scaring off rodents in order to reduce their numbers.

TO chemical deratization means relate rodenticides and their forms, synergists and repellents.

TO physical deratization means relate mechanical devices that kill rodents (crushers, traps, glue traps) or catching means of a single and multiple actions that restrict the movement of rodents (lizards), as well as repelling devices (ultrasonic and electromagnetic emitters, electrical devices).

Deratant means and recommended ways of their

applications must meet the following requirements:

– be effective with respect to target species;

– do not have repellent properties (except for

repellents);

– be safe for humans and non-target species,

which implies the presence of antidotes or appropriate measures

application.

Deratization agents are tested in laboratory conditions

and in the natural habitats of rodents (in actual conditions).

In laboratory conditions, the effectiveness of deratization

means are assessed with a single or group content

animals. In natural habitats of rodents specify

regimes of application and the effectiveness of deratization devices.

All works are carried out in accordance with the existing

requirements, instructions, approved norms and rules,

methods of keeping laboratory animals and testing

deratization means.

Requirements for experimental animals and their conditions

content. The effectiveness of deratization products is determined by

laboratory forms of rodents of target species - gray and black

rats, brown mice, gray voles - species that are

the main objects of deratization in populated areas. AT

descendants of wild rodents grown in

conditions of the vivarium. If there are no laboratory descendants in the vivarium

wild rodents allowed in-situ testing in natural

conditions or in a populated area.

3.2. Methods of research effectiveness chemical rodenticidal preparations

3.2.1. Requirements for the conditions of the efficiency evaluation experiment rodenticide means. Controlled indicators and results processing

The purity of the experiment to assess the effectiveness of rodenticidal

means depends on the maintenance of permanent conditions of detention animals throughout the experience. Laboratory room, where conduct experiments, should be isolated from other premises vivarium, have a natural light regime, a room temperature (20-22 ° C) and stable relative humidity (50-60%).

The composition and quality of the feed remain constant throughout the experience. When studying the effectiveness of poisoned baits as a control uses an alternative feed that does not contain rodenticidal agent. Oatmeal for this purpose.

Determination of the effectiveness of substances and concentrates of active substances (DV) are studied on the prepared standard poison bait used as a model, with the known concentration of the active substance. For standard poisoning bait as a food base use oatmeal.

Standard bait with a certain concentration of LW is prepared, mixing the grain basis with the concentrate of the active substance.

The required amount of concentrate (K) in bait calculated by the formula:

$$K = \frac{П \cdot ДВ_2}{DV_1}, \text{ where}$$

K is the amount of concentrate (g);

П - amount of bait (g);

$ДВ_1$ - content of DV in the concentrate (%);

$ДВ_2$ - the given maintenance ДВ in a bait (%).

During the experiment, animals are provided with free access to water and feed. Use tap water without foreign odor and taste with a pH of 6.8-7.0. Design automatic drinkers eliminates water pollution. Feeders with feed on the opposite walls of the cell - this reduces its pollution and dragging. Alternative feed and the bait is spread into different feeders of 100 g for rats and 50 g for mice. Complete eating of feed is not allowed; when eating 2/3

feed in the feeder add a new, up to the original mass.

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The feeders are swapped daily to exclude
Addiction of rodents to one feeding place.

In the experiment, healthy rodents, approximately the same age (2-6 months) and body weight (gray rats 150-280 g, house mice 20-30 g; gray voles of 18-28 g). If the task of special studies, then in the experiments do not use pregnant or lactating females.

Rodents sampled for experiments are not treated insecticides, anthelmintic preparations, places of their maintenance Do not treat with disinfectants.

For experiments, 6 individuals of the same species are used. When obtaining fuzzy results, experiments are repeated on 10 rodents.

Before the experiment of rodents for 3 days are able to adapt to new conditions of detention and feeding. To study the effectiveness of rodenticidal agents in experiments are conducted until the moment of death of the animal, but no more than 3 days for rodenticides acute action, 10 days - for second-generation anticoagulants and mixed-action poisons (vitamins D2 and D3) and 14 days - for anticoagulants of the first generation.

The effect of poisoned bait on rodents is determined by three main parameters:

– Eating (attractiveness) of bait;

– τηε number of dead;

ισ τηε time of death.

As additional parameters use the quantity absorbed by the rodent DV in milligrams and the amount of LW in

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recalculated per unit of body weight (mg / kg). Rodent weight and its change determine at the beginning of the experiment and at its end.

The primary results of the experiments are recorded in the "Workbook", and then calculate the necessary parameters. Examples of calculations and their The layout is shown in Fig. 3.1, in Table. 3.1 and 3.2.

Eating is determined by the amount of food eaten rodent for one day. For this purpose we weigh each day The remainder of the feed in the trough determines the difference between the initial weight of the feed and the weight of the remainder.

Fig.3.1. Working journal (sample)

No. n \ n	date start experience	d and AT	l about P	the weight (d)	Duration of the experiment (day)													
					0	1	2	3	4	5	6th	7th	8	9	10	11	12	13 14
				rodent (d)	265	-	-	-	-	-	245							
				remainder baits in feeder (d)	100	85	75	70	65	60	60							
		and I		eaten baits (g)	0	15	10	5		5	5	0						
1	3.03.05.	sa ser s R TO	eet himself	remainder an alternative feed in feeder (d)	100	90	85	80	75	75	75							
				eaten an alternative feed (g)	0	10	5		5	5	0	0						
				rodent (d)	27th	-	-	-	-	22								
				remainder baits in feeder (d)	50	43	37	32	29	29								
		ь w s	a to	eaten baits (g)	0	7th	6th	5	3	0								
7th	3.03.05.	th m at about m about D	himself	remainder an alternative feed in feeder (d)	50	45	43	40	38	38								
				eaten an alternative feed (g)	0	5	2	3	2	0								

The average rodent weight is calculated by the formula:

$V_{cp} = (V_x + v_x) / 2,$

Where:

V_{cp} - average rodent weight;

x is the rodent's serial number;

V_x - rodent weight at the beginning of the experiment;

v_x - Rodent weight on the day of death (Table 3.1).

Table 3.1.

The dynamics of the consumed bait and alternative
m gray rats

No. n \ n		Rat weight (g)			Number of food eaten by days (g)											
		at first experience	in a day doom	Average	1 ABOUT TO		2 ABOUT TO		3 ABOUT TO		4 ABOUT TO		5 ABOUT TO		6th ABOUT TO	
x		V x	v x	V cf	P x	p x	P x	p x	P x	p x	P x	p x	P x	p x	P x	p x
1	eet himself	265	245	255	15	10	10	5	5	5	5	5	0	†	†	
...	...	....	....	....	...	...	...	...	...	...	...	...	...	...	...	...
n	a to himself	200	196	198	15	15	15	5	10	5	5	0	†	†		
Average (m)		m Vx	m vx	$m_{Vsr} = \frac{\Sigma V_{cp}}{n}$ (1 ... n)												

Note: † - death of the rat; O - experience (studied bait); K - control (alternative feed); P_x - eaten food in the experiment (x = 1,2 ... n); p_x - eaten food in the control; n is the number of rats in the experiment; the rest of the notation in the text.

The arithmetic average (m) of the weight of the rats at the beginning of the experiment (mV_x) and the weights of the killed rats (mv_x) are calculated from the following formulas:

$$m_{Vx} = \frac{\Sigma V_{1 \dots n}}{n} \pm \Delta \text{ or } m_{vx} = \frac{\Sigma v_{1 \dots n}}{n} \pm \Delta,$$

Where:

n is the number of rats in the experiment (sample size);

$\Sigma V_{1 \dots n}$ is the sum of the weight of the rats, at the beginning of the experiment ($V_1 + V_2 + \dots + V_n$);

$\Sigma v_{1 \dots n}$ is the sum of the weight of dead rats ($v_1 + v_2 + \dots + v_n$);

Δ is the mean error.

$$\Delta = \frac{\sigma}{\sqrt{n}}, \text{ where}$$

σ is the standard deviation.

Table 3.2.

Eating of bait and amount of DV in the body of gray rats

No. n \ n	Eaten food during the experiment					amount active ingredient, absorbed by a rat		Time doom rats (day)
	in grams.			at %		mg	mg / kg	
x	Opst ΣP_x	Controlь Σp_x	The result about R	Opst G_x	Controlь R_x	DB_x	Dv_x	

								t_x
1	40	25	65	62	38	2.0	7.8	6th
.	.	.	.	.	.	.	.	.
n	45	25	70	64	36	2.3	11.6	5
average								

Note: the concentration of LW in the bait is 0.005%; designations in the text.

Determination of the relative consumption of the bait

G_x and an alternative feed R_x for the rat in% (Table 3.2):

$$G_x = \frac{\Sigma P_x}{P} \cdot 100\%, \qquad R_x = \frac{\Sigma p_x}{P} \cdot 100\%,$$

Where:

G_x - bait consumption (%);

R_x - consumption of alternative feed (%);

ΣP_x - amount of bait eaten by the rat in the experiment (mg);

Σp_x - the amount of alternative food eaten by a rat in

experiment (mg);

P is the total amount of feed (mg) eaten by the rat in the experiment ($\Sigma Px + \Sigma px$);

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Definition quantities acting substances,

absorbed by the rat in mg (Table 3.2):

$$DB_x = \Sigma P_x \cdot 10 \cdot C,$$

Where:

DB_x - the amount of active substance absorbed

rat in mg;

C is the concentration of the active substance in the bait (%);

Definition quantities acting substances,

absorbed by a rat in terms of kilogram weight (Table 2):

$$Dв_x = \frac{1000 \cdot DB_x}{Vsp},$$

Where:

DB_x - the amount of active substance absorbed

rat in mg / kg;

V_{cp} is the average weight of the rat (Table 3.1).

3.2.2. Methods for investigating the effectiveness of various forms of rodenticides

Study of the effectiveness of finished forms of rodenticides

acute and cumulative effects.

Ready-made forms of rodenticides can be testing in the form of grain bait, granules, soft and paraffin briquettes, capsules, biscuits, tablets, pastes, etc. with a known active substance and its concentration.

The effectiveness of finished forms of rodenticides is studied in target species of rodents: gray rats, brown mice, gray voles. In the course of the experiment, a comparative analysis

eaten poison bait and alternative food, determine the timing of the death of rodents and their number in percent, of the total number of rodents used in the experiment (paragraph 3.1).

The duration of the test for the test finished form with rodenticide of different types of action is given in § 3.1.

When the ready rodenticidal form is low rodents do not die within the specified time.

The criteria for evaluating the effectiveness of prepared forms are the following parameters:

– for rodenticides of acute action:

death in the presence of alternative feed of mice and rats (%) - not less than 80;

time of death (day) - no more than 3;

eaten the finished form of rodenticide or cooked standard poison bait in the presence of an alternative feed mice and rats (% of the daily diet) - not less than 10%;

– for anticoagulants of the first generation:

death in the presence of alternative feed of mice and rats (%) - not less than 80;

time of death, (day) - no more than 14;

eaten the finished form of rodenticide or cooked

standard poison bait in the presence of an alternative
(except for baits for mice based on ethylphenacin,
zookoumarin, kumatetralyl) in mice and rats (% of daily
ration) - not less than 15;

eaten the finished form of rodenticide or cooked
standard poison bait based on ethylphenacin, zookoumarin,

kumatetralyl in the presence of alternative food in mice (% of
daily ration) - not less than 20;

– for anticoagulants of the second generation:

death in the presence of alternative feed of mice and rats (%) - not
less than 90;

time of death (day) - no more than 10

eaten the finished form of rodenticide or cooked
standard poison bait in the presence of an alternative feed
mice and rats (% of the daily ration) - not less than 15;

– for ready-made forms with vitamins D_2 and D_3:
death of mice (%) - not less than 80;
rats (%) - " -";
time of death (day) - no more than 7;
eatability of the finished form in the presence of alternative food in mice
(% of the daily diet) - not less than 10;
rats (% of the daily ration) - " -".

Investigation of the effectiveness of substances (technical DV)
and DW concentrates.

Substances and concentrates of DV are studied in the form of a standard
poisoned bait (item 3.1).

On the basis of the substance, concentrate is first prepared with the desired
the content of DV in the form of an oily or other solution, an emulsion,
suspension or powder with a dye sufficient to indicate
the uniformity of the distribution of the active substance in the bait.
As a colorant, substances that do not affect
eating of bait rodents. Number of active

substances in the concentrate are calculated by the formula:

$$ДВ = \frac{к \cdot ДВ_2}{ДВ_1},$$

Where:

DV - the amount of active ingredient (g);

k is the amount of concentrate (g);

$ДВ_1$ - content of DV in substance (%);

$ДВ_2$ - the given maintenance ДВ in a concentrate (%).

To prepare the standard bait used in

As a model, the concentrate is mixed with the food base

(oat groats). The quality of mixing is controlled by

uniformity of coloring bait dye.

Assessment of the effectiveness of substances and concentrates is carried out on

standard bait, according to the methodology and criteria set out

in Section 3.1.

Investigation of the effectiveness of sticky rodenticidal

coatings.

The effectiveness of a sticky rodenticidal coating at

of the given concentration of DV are studied on the rodents of the target species:

gray rats, brown mice, gray voles. Estimate the terms and

the percentage of rodent death, their relation to the sticky rodenticidal

cover, including the presence and nature of the avoidance reaction

(neophobia, repellency).

Sticky rodenticidal coatings are studied on rodents,

contained singly in cages, where two

of the same track or tunnel for their movement from

nesting house to the trough. After adaptation of the rodent to

conditions of maintenance, per track or tunnel length 0.25 m

Through the entire length, a sticky rodenticide coating is applied in 1-2 mm. Another track or tunnel remains clean.

Mark the number of rodent passes to the stern for each track for a day. Observations are carried out visually during the period maximum rodent activity or by using automatic sensors installed in front of the trough. The time of death depends from the number of passes of the rodent on a toxic sticky coating.

The rodent is allowed to contact the sticky rodenticide coating no more than 10 days. If he does not was killed, he was caged in a cage to determine the time of death.

The coating is considered effective if at least 80% Experimental animals die within 10 days.

If within the first two days animals use only track or tunnel without coverage, then there is a reaction neophobia to cover. If this reaction persists to the end exposure of a sticky rodenticidal coating, then repellency.

The effectiveness of sticky rodenticidal coatings in the absence of an alternative path to the conditions of forced passage of the rodent through the tunnel, treated with a sticky rodenticidal coating, which allows determine the number of passes necessary for his death.

Criteria for assessing the effectiveness of sticky rodenticidal covers the following parameters:

death of mice and rats (%) - not less than 80;

Exposure (day) - no more than 10.

3.3. Methods for investigating the effectiveness of repellents

A study of the effectiveness of deterrent chemical compounds for eating food containing repellent.

The effectiveness of repellents is studied in rodents targeted

species: gray rats, brown mice, gray voles.

The insect repellent is introduced into the feed. With different concentrations of repellent in the diet determine the presence or the absence of a deterrent rodents and the degree of its repellent activity. To this end the same amount of food with a repellent and without it offer rodents contained alone. As a food base use both in the experiment and in the control of oatmeal (paragraph 3.1). AT for 3 days take into account the food consumption with repellent and without him.

On basis of received the results define the coefficient of repellent action of feed with repellent to formula:

$$CODE = 100 - \frac{100 \cdot T_c}{(T_c + E)},$$

Where:

CODE is the repelling factor (%);

TS - eaten food with content of repellent C (r);

E - eaten food without repellent (d);

The effectiveness of the repellent is evaluated by RCD.

COD = 100 ~ 90 - strongly repelling substances;

COD = 89 ~ 80 - substances with satisfactory results repelling;

COD = 79 and below are ineffective substances.

Duration of the repellent activity of a chemical substance determine by the CODE of the feed with the additive of repellent.

A study of the effectiveness of deterrent chemical connections to choose the path to food.

The experiment is carried out in a cell divided into two halves a partition with two holes. Rodent from the nest half cells tends to penetrate through this or that hole half, where there is food and water. In the experiment, one of the

The holes are covered with a screen of filter paper,
treated with a solution of repellant, the other - close
an untreated screen of filter paper. Screen
replace with new one at each damage. Observations for
the behavior of the rodent is carried out daily for two hours for three
days in the daytime. Mark the amount of damage
screen of each hole and transitions of the rodent into the stern
half. The effectiveness of the repellent is evaluated by the "coefficient
damage "(CP). The coefficient is determined by the ratio of the number
damage to the rodent of a hedge treated with repellent ($П_р$) к
the number of rodent hedges not processed
repellent (P_c).

$$CP = \frac{P_r}{P_c}$$

If the KP is less than one, the test substance possesses
repellent properties. The highest repellent activity
chemical compounds, the "damage coefficient"
which approaches or is zero. The test substance is not

has repellent properties if the KP is equal to or greater than
units.

Study of the effectiveness of ultrasonic rodenticidal
means for their effect on feed intake.

Determination of the efficiency of an ultrasonic radiator
(Ultrasound) is carried out in a specially equipped test site,
size 5x10 m, divided into pilot and control
half a metal barrier-screen. In each half
expose an equal number of shelter houses and feeders such
way that one half of the polygon is a mirror
reflection of the other. Feed in the feeder should always be in
excess.

In the experimental half, place an ultrasound device so that
The feeders were exposed to ultrasonic radiation. Then
In the pilot and control half of the test site, equal

group of rodents of 3-5 individuals in each group. Within three
Rodents adapt to new conditions. In the registration
daily, the amount of food they eat is
each half of the polygon. At the end of the adaptation period,
The experimental half of the polygon includes an ultrasound device. Device
remains on for 5 days. During this time in the experimental and
control half of the polygon determine the daily
feed consumption by rodents.

Results are recorded in the "Workbook", and then count
coefficient of deterrent ultrasound. Examples of calculations and their
registration are presented in Table. 3.3.

The effectiveness of ultrasound for rodents is assessed by
fodder consumption. Compare the amount of food eaten

control and experimental groups. The ultrasound device is evaluated as
Effective if during its operation, the food is eaten
animals experienced groups amounted to not more 10%
average daily ration, and the difference in feed intake with
the control group is at least 40%.

The range of ultrasound is determined experimentally,
installing feeders at different distances from the source
radiation.

Table 3.3.

Eating grains by gray rats when exposed to ultrasound

No.	The amount of food eaten (g)															
	in the period of adaptation (day)						in the experiment (day)									
	1	2	3	Total	more Wed		1	2	3	4	5	Total	more Wed	Total	at percentages	the tortures about control
1	28	25	15	68	23.7		4	1	2	2	5	14				
2	25	23	thirty	78	26.0		3	1	1	6th	4	15				
3	20	20	27th	67	22.3		2	3	1	3	6th	15				
4	12	16	13	41	13.7		1	1	1	9	7th	19				
5	thirty	21	20	71	23.7		2	1	2	6th	5	16				
6th	27th	20	17th	64	21.3		1	2	1	9	8	21				
Total	142	125	122	389	129.7	13		9	8	35	35	100	20	149.7	13	87

average						64.8												
a		1	19	23	24	66		24	thirty	22	25	29	130					
wines lo	and I	2	21	22	21	64		23	26th	31	29	23	132					
th on	5	3	19	21	23	63		26th	24	23	33	23	129					
flax	sa ser s R TO	4	17th	20	22	59		20	27th	27th	thirty	21	125					
		5	20	25	21	66		27th	21	21	31	20	120					
ontro TO		6th	16	24	23	63		22	22	20	24	14	102					
total			112	135	134	381	127	142	180	144	172	130	738	147.6	274.6	54	46	
average						63.5							123					

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3.4. Methods for investigating the effectiveness of mechanical deratant means

Study of the effectiveness of sticky masses

and ready-made glue traps.

The test is carried out in cages with a single or group the content of rodents. Determination of the effectiveness of adhesive masses are carried out by means of glue plates made of material that does not absorb glue. When the rodent contacts the glue the surface of the plate, the adhesive mass must hold the animal. For house mice and voles, plates of size 12x20 cm. For experiments with rats, plates of size 20x25 cm. Adhesive mass on the surface of the plate is applied continuous layer not more than 1 mm. Plates with glutinous mass placed in front of the trough, or elsewhere and then to the center glue surface is glued as a small bait a piece of bread with vegetable oil.

The indicator of the effectiveness of adhesive is keeping not less than 80% of small animals weighing not more than 100 g.

Investigation of the effectiveness of the animal.

Panting for rats and mice come entirely from metal mesh, galvanized iron with holes, from metal mesh, fixed on a wooden board or plastic. Disposable devices have a door that slams when the rodent pulls the bait off the hook or pressing on the trapezoid. Balancing one-off swagger

consists of a plastic tube of square cross section, curved
at a certain angle, and the door slamming at
violation of balance. The rodent enters inside the trap by

horizontally located part of the tube and on going to
Another half of it breaks the balance of the trap, from which the door
Rises and slams.

Reusable animals (such as tops) are made of
metal mesh. Rodents fall inside by tapering
passage or through a hole with a trap. Trapik works under
the weight of the rodent, which falls into the top, and the
The action of the spring assumes its original position and
closes the exit rodent. Assessment of the effectiveness of the animals
are carried out according to the procedure used for the test of single-use
mechanical devices taking into account the specificity of the reusable
hit.

3.5. Methods for studying the effectiveness of chemical rodenticides in conditions of group maintenance of rodents

Experiments on the family or artificially created
grouping of rodents allows to simulate their behavioral
reactions with respect to rodenticidal agents, characteristic
for natural conditions, and more accurately assess the effectiveness of
tested means.

Study of the effectiveness of finished forms of rodenticides
acute and cumulative effects.

The reaction of rodents to rodenticidal agents in conditions
group content in the enclosure approximately reflects their
behavior in natural conditions. Relationships of animals in
grouping is determined by a different degree of rodent contact with
rodenticidal agents than with a single content,
related to their social behavior.

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Experiments to assess the effectiveness of rodenticidal funds on family or artificially created groups of rodents are carried out in enclosures equipped with one or two domes. kami, a drinker and two feeding troughs that are hung on one of the side walls of the cage or strengthen symmetrically on opposite walls. Grouping is formed from 5 rodents (three males and two females, and an increase in the number of females probably, and males - is not recommended) and withstand them together within 3 days - to establish a hierarchical structure in the grouping (the minimum time required), and To determine the amount of food eaten per day. After education in the grouping of sustainable relationships, one of the feeders fill with poisoned bait, another - an alternative (control) food. Weight it in the troughs should exceed the daily allowance required for nutrition group, in 1,5 times. As necessary, add food to the feeders. To determine the amount of food we weigh the remainder of the feed (paragraph 7.1.). Feeders change places on a daily basis.

The criteria for evaluating the effectiveness of prepared forms are parameters specified in clause 7.2.2.

Investigation of the effectiveness of sticky rodenticidal coatings.

The experiment is carried out in an enclosure with 1-2 nesting house and drinker. The rodent grouping is formed as described above (page 13).

Sticky rodenticidal coating is applied a layer of 1-2 mm on a plate measuring 25x25 cm. In the middle of the plate, small feeder (no more than 6x6 cm) with the weight of the feed,

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exceeding the daily diet of rodents. The plate with feeder is placed in the enclosure. Food is used as a bait, and eating it as an indicator of rodents contact with

sticky rodenticide coating. The balance is weighed daily
feed in the trough and determine the feed intake. Exist
direct relationship between the number of rodent contacts with
sticky rodenticide coating and the amount eaten
stern.

The time of death of rodents depends on the number of contacts they have with sticky rodenticide coating. Experiment in the cage spend no more than 10 days. Then the surviving rodents transplant one by one into the cells to determine the timing of their death.

To determine the repellency of the sticky rodenticidal cover in the enclosures are placed two feeders with the same food. One feeder is fixed on a site treated with poisonous coating, the other - on the site without coverage. In count eaten food in each trough is determined by the presence of coatings of repellent properties.

3.6. Methods for assessing the effectiveness of deratization products in The natural habitats of rodents (in actual conditions)

3.6.1. Requirements for testing deratization means in full-scale conditions

Biological activity and target efficiency

Deratization means are studied at sites populated rodents. To conduct experiments, select objects with high or medium numbers of rodents.

The presence and number of rodents, as well as their species composition, initially established on the basis of a survey of workers object and visual him surveys by traces vital activity of rodents. Specification of species composition of rodents and the degree of population of the object are carried out with the help of control-dust (trace) areas or food baits without toxicant, that is, methods that do not change the composition and the number of rodents on the site.

Study of the effectiveness of deratization equipment on the site

perform no more than 10 days for rodenticide acute
action, mechanical means (traps, animal, glue
devices) and ultrasonic radiators (ultrasound), 28 days for
anticoagulants of the second generation and rodenticides of mixed
actions (vitamins D_2 and D_3) and 40 days for anticoagulants
first generation.

Effectiveness of the poison bait on rodents
determined by the following main parameters:

– eaten bait;

– time of appearance of corpses;

– change in the relative number of rodents

– speed of release of the object from rodents.

On the object, the poisoned bait is placed in the feeding troughs,
depending on the number of rodents in the amount of 20 to 100 g.
At an object of less than 100 m^2, no more than five feeders are placed.
Simultaneously equip no more than 10 control-dust
(trace) sites.

On sites more than 100 m^2 feeding troughs
in accordance SanPin 3.5.3.1129-02, that is, 8 g of bait per 1 m^2 .

In this case, one feeder should account for 50 g of the poisoned
baits.

Additional parameters by which
assess the effectiveness of deratization products in natural
habitats of rodents are: decrease in their number and
dynamics of species composition, and for mechanical or glutinous
deratization means - their catchability.

Eating is determined by the amount of food eaten
animals on the site for one or two days. To this end
weigh the remainder of the feed in the feeders and determine the difference
between the initial weight of the feed and the weight of the remainder. Frequency
control weights depends on the number of rodents in the
object. At high numbers, weighing the feed should be
carry out once a day. Feeders can serve

plastic disposable plates, boxes of 10x10 cm,

special standard containers for bait.

Simultaneously, by the method of control-dust (trace)

The relative abundance of rodents in

object. Control of the number of rodents before and after the experiment

are carried out according to the same procedure with observance of standard

requirements for the arrangement of inventory and the timing of their exposure

(that is, the period between the arrangement and control of accounting devices).

The speed of object release is determined by the increase

the number of non-tracked control-dust (trace)

sites by days.

The primary results of the experiments are recorded in the "Working Journal"

(Figure 7.2).

At the site chosen for testing,

other deratization activities, as well as

there must be other rodenticidal agents.

In the process of testing deratization devices are evaluated

also the safety and convenience of their use in natural

habitats of rodents and determine compliance with the rules

occupational safety and health.

Fig. 3.2.

Working journal

No. n / surface adki	No. fodder shki	date start experience	d and AT	Name control accounting	Time of the control account (day) 0	2	4	6th	8	10	12	14	16	...
				the remainder of the bait in feeder (d)										
-	2	3.03.05.	-	eaten bait (d)										
			and I	Number of dust pads										
3	-	3.03.05.	sa ser s R TO	n / a platform with traces										
				n / playground without tracks										
			and J all abou m s abou D	Number of dust pads										
7th	-	3.03.05.		n / a platform with traces										
				n / playground without tracks										

3.6.2. Methods for investigating the effectiveness of deratization means

The technique of control and dust (trace) sites.

This technique allows you to detect rodents at the site and assess the population of its rodents, determine their species belonging and pathways movement. When learning deratization means and evaluation of their effectiveness, methodology control-dust (trace) sites are used for determination of the tendency of changes in the number of rodents at the site. The dynamics of sites monitored by rodents in the direction of decreasing

is due to means and reflects it efficiency.

Controlling-dust (trail) platforms measuring 10x20 cm covered with a layer of flour, talc or other pulverized material place in the places of probable movement of rodents by the object and, first of all, in corners, along walls, partitions, near doors and windows. The number of sites on the site depends on its area.

At the sites or premises of at least 100 m^2 equipped with no more than 10 sites. In this case, the sites are located along the skirting boards along the the sides of the doorway (two), in the corners of the room (four), under the window (one) and in the middle of the base of the side walls (two). At the facilities an area of more than 100 m^2 control and dust (track) sites equip from the calculation: one site for 5-10 m^2 .

In the tracks left by the rodents on the control dust (trace) sites, calculate their relative number by object. To do this, determine the number of sites with traces stay rodents and calculate the percentage of traced sites according to the formula:

$$K = \frac{A \times 100}{B},$$

Where:

K is the number of traced sites in%;

B - all sites on the site (pieces);

A is the number of tracked sites (pieces).

If traces of rats, mice or
voles to determine their relative number,
Calculate the% of the traced sites for each species.

Study of biological activity and target
the effectiveness of deratization products in natural places
a habitat.

Biologically active agent should cause death
rodents (poison baits, sticky coatings, mechanical and
glue sacrificial devices), or restrict them
Movement (piercing) or penetration (repellents and ultrasound)
on the object. The faster the rodents disappear in the premises of the object,
the more effectively the test medium and the less
sites is tracked by rodents. Efficiency deratization
the means are assessed by the rate of release of the object from rodents.
For this, before the experiment is started,
tracked and not tracked sites and count
the relative number of rodents. Then the object is placed
tested deratization means in accordance with the norms,
specified in clause 7.1. After that, daily check-
dust (trail) sites, given the number of tracked and
unresolved sites.

The consumption of baits is determined by the amount of food,
eaten by rodents on the site for one or two days.
The percentage of the bait consumed is determined from the total
for each day or on average for the selected
period. To determine the amount of food eaten in the trough
lay out weighted portions thereof. In each trough
spread out from 50 to 100 g of bait and arrange as indicated in
item 7.1. In order not to miss feeders when inspecting, they should be
to number.

After one or two days, the food in the feeding troughs is weighed. After that, all the remaining food is harvested, and in the feeding troughs lay out another of the previously prepared and weighted new portion. Subtracting from the initial weight of the feed following, determine the mass of food eaten by rodents for day in each feeder. Food is laid out 2-3 times and average consumption of bait for a certain observation period.

Number of control dust (track) sites calculated on the basis of the norms indicated above.

Effectiveness of pressure, live, sticky masses or ready-made adhesive traps, sticky toxic coatings, ultrasonic rodenticidal means, repelling chemical connections are evaluated by the rate of release of the object from rodents, using for control the method of control-dust (trace) sites.

The effectiveness of deratization equipment is calculated by formula:

$$E = \frac{(K_1 - K_2) \times 100}{K_1},$$

Where:

E - the effectiveness of the deratant (%);

K_1 - the number of tracked sites on the site before the start experiment (%);

K_2 - number of sites at the end of the experiment (%).

K_1 and K_2 determined by the percentage formula sites - K (item 7.2)

3.6.3. Criteria for assessing biological activity and target the effectiveness of deratization products in natural places a habitat

– for rodenticides of acute action:

eaten the finished form of rodenticide or cooked standard poisoned bait by mice and rats (% of total bait) - not less than 5;

the appearance of corpses of rodents (day) - 3;

decline quantities tracked sites or relative numbers of rodents by 98% (day) - within 10;

– for anticoagulants of the first generation:

eaten the finished form of rodenticide or cooked standard poison bait (except for baits for mice on based on ethylphenacin, zookoumarin, coumatetralyl) in mice and rats (% of total bait) - not less than 15;

the appearance of corpses of rodents (day) - by 10;

decline quantities tracked sites or relative abundance of rodents by 98% (day) - within 40;

eaten the finished form of rodenticide or cooked standard poison bait based on ethylphenacin, zookoumarin, kumatetralil mice (% of the total bait) - not less than 10;

the appearance of corpses of rodents (day) - by 15;

decline quantities tracked sites or relative abundance of rodents by 98% (day) - within 40;

– for anticoagulants of the second generation:

eaten the finished form of rodenticide or cooked standard poison bait in mice and rats (% of total amount of bait) - not less than 10;

the appearance of corpses of rodents (day) - by 10;

decline quantities tracked sites or the relative abundance of rodents by 98% (day) - for 28;

– for ready-made forms with vitamins D_2 and D_3:

eatability of the finished form by mice (% of total

bait) - not less than 10;

rats (% of total bait) - "-";

appearance of corpses of mice (day) - by 5;

decrease in the number of sites monitored or relative the number of rodents by 98% (day) - during 28;

– for sticky rodenticidal coatings:

the appearance of corpses of rodents (day) - by 15;

decline quantities tracked sites or relative abundance of rodents by 98% (day) - within 40;

– for presses, vivacity, adhesive masses and ready-made glue traps:

decline quantities tracked sites or relative numbers of rodents by 98% (day) - within 10;

– for ultrasonic repelling radiators:

decline quantities tracked sites or relative number of rodents by 98% (day) - during 1-2 days of emitter operation;

– for chemical repellents:

decline quantities tracked sites or relative number of rodents by 98% (day) - during 1-2 days of at least 5.