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PAKISTAN STANDARD SPECIFICATION

FOR

HAIR CREAM

PAKISTAN STANDARDS AND QUALITY CONTROL AUTHORITY,
STANDARDS DEVELOPMENT CENTRE,
Plot No. ST-7A, Block-3, Scheme 36, Gulistan-e- Johar
Karachi.

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0. Foreword

0.1 This Pakistan Standard was adopted by the Pakistan Standards Institution on 25.10.2001 after the draft finalized by the Cosmetic & Toilet Goods Sectional Committee had been approved by the Chemical Divisional Council.

0.2 Hair Creams are intended to provide to the hair goods grooming, luster and some degree of hair conditioning. The major property required for conditioning is moisturing. Moisture must be added for this purpose by direct application of water is of little benefit since evaporation is equally rapid and equilibrium is as soon reached. A men has to be provided to prevent the absorbed water from evaporating and emulsions of oil and water prove useful in this respect.

0.3 This Standard is intended chiefly to cover the technical provision relating to the supply of material and it does not include all necessary provisions of a contract.

0.4 In the preparation of this standard specification the views of the representative from the manufacturers, research institutions and consumers were sought. In addition the valuable assistance derived from Sri Lanka Standard SLS: 611 “Hair Cream” is acknowledged with thanks.

0.5 For the purpose of deciding whether and particular requirement of this standard is complied with the final value observed or calculated expressing the result of a test or analysis, shall be rounded off in accordance with PS: 103 – 1996 Rules for rounding off numerical values. The number of significant places retained in the rounded off values should be same as that of the specified value in this standard.

1. SCOPE

This specification prescribes the requirements and methods of sampling and test for hair creams. These include water-in-oil and oil-in-water emulsions. The specification does not cover hair oils, brilliantines and pomades.

2. REQIREMENTS

2.1 General Requirements

The hair cream shall be in the from of an emulsion. It shall be of uniform texture, free from extraneous matter and may be perfumed.

2.2 Ingredients

unless specified otherwise, all raw materials used in the manufacture of hair creams shall conform to the requirements prescribed in the relevant Pakistan Standards and where such standards do not exists, shall be herpetologically safe.

2.2.1 The dyes, if used, shall comply with the provisions of PS:_____

2.2.2 Ingredients other then dyes shall comply with the provisions of PS:_____

2.3 Other requirements

The hair cream shall comply with the requirements given in Table 1 when tested according to the relevant methods given in Column 4 of the table.1

TABLE 1 REQUIREMENT FOR HAIR CREAM

S.#	Characteristic	Requirement	Method of Test (Ref. to Cl # In Appendix C)
1	2	3	4
i.	Thermal stability	To pass the test	C.2
ii.	pH at 27 ± 2°C	5.0 to 9.0	C.3
iii.	Total non-acqueous content, percent by mass, min	15	C.4
iv.	Water content, percent by mass max	70	C.5
v.	Peroxide Value, milli equivalents/Kg, max	10	C.6

2.3 Microbiological Limits

2.4 The material shall also comply with the microbiological limits given in Table 2, when tested according to the relevant methods given column 4 of the table.

TABLE 2 MICROBIOLOGICAL LIMITS

S.#	CHARACTERISTIC	LIMIT	METHOD OF TEST (Ref to Cl. No in Appendix C)
1	2	3	4
i.	Number of micro organisms per gram, max	1000	C.7
ii.	Pseudomonas aeruginosa	Absent in 10 g	C.7
iii.	Staphylococcus aureus	Absent in 10 g	C.7

3. PACKING AND MARKING

3.1 The hair cream shall be packed in suitable containers.

3.2 The containers shall be legibly and indelibly marked with the following:

a. Name of the product;
b. Name and address of the manufacturers
c. Net mass in grams or net volume in millilitres; and
d. Batch or code number
e. Date of manufacture
f. Date of Expiry.

4. SAMPLING

Representative samples of the material shall be drawn as prescribe in Appandix A

5. METHODS OF TEST

Tests shall be carried out as prescribed in Appendix C

6. CONFORMITY TO STANDARD

A lot shall be declared as conforming to the requirements of this specification; if the following conditions are satisfied.

6.1 Each containers examined as in A.6.1 satisfies the relevant requirements.

6.2 The composite sample tested as in A.6.2 and A.6.3 satisfy the relevant requirements.

APPENDIX A

A. SAMPLING

A- 1 Lot
In a consignment all the containers containing hair cream representing the same batch of manufacture shall constitute a lot.

A – 2 GENERAL REQUIREMENTS OF SAMPLING
In drawing, preparing, storing and handling sample, the following precautions shall be observed.

A- 2. 1 Samples shall be drawn in an environment not exposed to damp air, dust or soot.

A – 2.2 A sampling tube may be used for drawing the materials form the containers. It shall be clean and dry when used.

A-2.3 The sample shall be placed in clean, dry, glass or any other suitable containers. The sample containers shall be sealed air-tight after filling and shall be marked with necessary details of sampling.

A-2.4 The material being sampled, the sampling instrument and the sample containers shall be protected from adventitious contamination.

A-2.5 Samples shall be stored so that the conditions of storage do not affected the quality of the material.

A-2.6 When drawing samples for microbiological examination in addition to the requirements specified in A.2.1 to A.2.5, the following precautions shall be observed :

A.2.6.1 Samples shall be drawn under aseptic conditions.

A.2.6.2 The sampling instrument and sample containers shall be sterilized using an appropriate method.

A.2.6.3 If storage is necessary, the samples shall be stored at room temperature and testing shall be carried out as soon as possible.

A.3 SCALE OF SAMPLING

A.3.1 Samples shall be tested from each lot for ascertaining the conformity of the material to the requirements of this specification.

A.3.2 The number of containers to the selected from the lot shall be in accordance with Table 3.

TABLE 3 – SCALE OF SAMPLING

No of containers in the lot (1)	No of containers to be selected (2)
Up to 50	3
51 to 200	4
201 to 400	5
401 to 650	6
651 to 900	7
901 to 1 200	8
1 201 to 1 500	9
1 501 and above	10

A.3.3 The containers shall be selected at random. In order to ensure randomness of selection, random number tables as given in PS: 1720 – 1985

A.4 CXOMPOSITE SAMPLE

A.4.1 An equal quantity of material shall be drawn from the top, middle and bottom portions of each container selected as in A.3.2 with the help of a sampling instrument. The material so obtained shall be mixed thoroughly to form a composite sample which shall be of a size sufficient to carry out tests for all requirements specified in A.6.2

A.4.2 A separate composite sample of about 20 g shall be prepared under the conditions specified in A.2.6 for testing microbiological requirements before the preparation of a composite sample for testing the other requirements.

A.5 REFERENCE SAMPLE

If a reference sample is required for requirements other than the microbiological requirements, the size of the composite sample shall be three times as much as the amount required in A.4.1. The composite sample so obtained shall be divided into three equal parts, each forming a composite sample. One for the purchaser, another for the supplier and third as the reference sample which shall be used in case of dispute between the purchaser and supplier.

A – 6. NUMBER OF TESTS:

A-6.1 Each container selected as in A.3.2 shall be examined for packaging and marking requirements (see 3)

A-6.2. Tests for all requirements other than microbiological requirements shall be carried out on the composite sample prepared as in A.4.1.

A-6.3 Tests for microbiological requirements shall be carried out on the composite sample prepared in A.4.2.

APPENDIX B
LIST OF RAW MATERIAL CONVENTIONALLY USED IN FORMULATION OF HAIR CREAM

B.1 EMULSION FORMERS

a) Mineral Oils and
b) Vegetable Oil, for example, castor oil, olive oil, etc.

B.2 EMULSION

a) Be eswax;
b) Carnauba wax;
c) Borax:
d) Proprietory synthetic emulsifiers; and
e) Soaps based on magnesium, calcium or triethanolamine.

B.3 CHELATING AGENTS

a) Sodium salt of EDTA; and
b) Sodium polyphosphates.

B.4 PRESERVATIVES AND ANTIOXIDANTS

a) Ethyl, propyl, methyl and butyl parahydroxy benzoates;
b) Gallic acid;
c) Octyl and dodecyl gallate;
d) Alpha tocopheryl acetate; and
e) Monoctadecyl ester of carboxymethyl mercapto succinic acid.

B.5 INORGANIC SALTS

a) Magnesium sulphate

B.6 EMOLLIENTS

a) Lanolin, lanolin esters, lanolin oils;
b) Fatty acid esters;
c) Alkanolamides; and
d) Petroleum jelly

B.7 THICKENING AGENTS

a) Carbopol;
b) Sodium alginate; and
c) Sodium C M C

B.8 HAIR SETTERS

a) Gum tragacanth;
b) Gum karaya; and
c) Shellac (water-soluble)

B.9 OTHER GROUPS OF INGREDIENTS

a) Perfumes;
b) Bactericides or bacteriostat; and
c) Dyes

APPENDIX C

METHOD OF TEST FOR HAIR CREAMS

C.1 QUALITY OF REAGENTS

C.1.1 Unless specified otherwise, chemicals of a recognized analytical grade and distilled water shall be employed in tests.

C.2 THERMAL STABILITY

C.2.1 Apparatus

A humidity chamber controlled at 60 percent to 70 percent relative humidity and 40 ± 1 ^{o}C.

C.2.2 Procedure

Spread a 20 mm broad, 5 mm thick strip of the material on the internal wall of a beaker. Keep the beaker for 8 hours in the humidity chamber between 60 percent to 70 percent relative humidity and 40 ± 1 ^{o}C.

C.2.3 The cream shall be considered to have passed the test if no oil separation is observed on removal from the humidity chamber.

C.3 pH at 27 ± 2 ^{o}C

C.3.1 Apparatus

A pH meter, preferably equipped with a glass electrode.

C.3.2 Procedure

C.3.2.1 For oil-in-water emulsion creams

Weigh to the nearest 0.1 g, approximately 5 g of the material into a 100 ml beaker. Add 45 ml of water and disperse the cream in it. Determine the pH of the suspension of 27 ± 2 ^{o}C using the pH meter.

C.3.2.2 For water in oil emulsion cream

Weigh to the nearest 0.1 g approximately 10 g of the material and add 90 ml of rectified spirit previously adjusted to ph 6.5 to 7.0 Warm if necessary, to 45 ^{o}C and stir thoroughly for 15 min. Filter the alcoholic layer through a filter paper and measure the ph of the filtrate at 27 ± 2 ^{o}C using the pH meter.

C.4 TOTAL NON-AQEOUS CONTENT

C.4.1 Principle of the method

The emulsion is broken up with dilute mineral acid and the fatty matter is extracted with ethyl ether. It is weighted after removal of the solvent.

C.4.2 Reagents

C.4.2.1 Hydrocholric acid, dilute 1 : 1 (v/v)

C.4.2.2 Ethyl ether.

C.4.2.3 Methyl orange indicator solution
Dissolve 0.1 g of methyl orange in 100 ml of distilled water.

C.4.2.4 Sodium sulphate, desiccated

C.4.3 Procedure

C.4.3.1 Weigh to the nearest 0.1 g, approximately 2 g of the material into a conical flask, add 25 ml of dilute hydrochloric acid, fit a reflux condenser to the flask and boil the contents until the oil and water phases separate. Pour the contents of the flask into a 300 ml separating funnel and allow it to cool to 20 ^{o}C. Rinse the conical flask with 50 ml of ethyl ether in 10 ml portions and pour the ether risings into the separating funnel. Shake the separating funnel well and leave until the layers separate. Separate out the aqueous phase and wash with 50 ml portions of ether twice. Combine all the ether extracts and wash them with water until free of acid (test with methyl orange indicator solution). Filter the ether extracts through a filter paper containing sodium sulphate into a conical flask which has been previously dried at a temperature of 60 ± 2 ^{o}C and then weighed. Wash the sodium sulphate on the filter paper with ether and dry the material remaining in the flask at a temperature of 60 ± 2 ^{o}C till the difference between two successive weighing does not exceed 1 mg.

C.4.4 Calculation

Total non-aqueous content, percent by mass = $100 \times \frac{m_1}{m_2}$

Where

m_1 = mass, in grams, of the residue; and
m_2 = mass, in grams, of the test portion.

C.5 WATER CONTENT

C.5.1 Apparatus

The apparatus, shown in Fig. 1 consists of the following;

a) Flask, 500 ml capacity, made of hard resistant glass;

b) Trap, the cylindrical portion of the receiving tube is 146 mm to 156 mm in length, graduated to contain a volume of 10 ml and is sub divided into 0.1 ml divisions; and

c) Condenser, approximately 400 mm in length and the bore diameter of the inner tube is 16 mm to 17 mm. The condenser is connected to the trap as shown in the figure 1

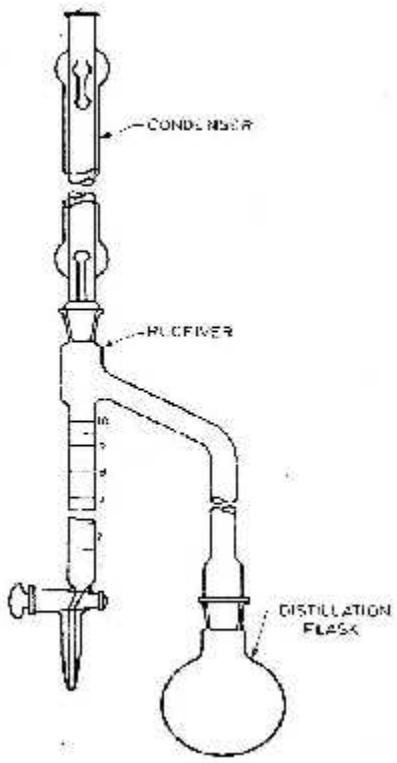

FIGURE No 1 – Entrainment distillation of Water

C.5.2 Reagents

C.5.2.1 Tolume, treated with excess water and distilled

C.5.2.2 Heptene; and

C.5.2.3 Xylene.

C.5.3 Procedure

Weigh to the nearest 0.1 g approximately 10 g of the material into the flask. Add about 200 ml of the solvent and a few pieces of dry pumice stone. Connect the apparatus and fill the receiving end of the trap with the solvent, poured through the top of the condenser. Heat the flask gently for 15 min, and then the solvent begins to boil, reflux at a rate of 2 drops per second until most of the water has passed. Increase the rate to about 4 drops per second. When the water has apparently all distilled, rinse the inside of the condenser tube with solvent while brushing down the tube with a tube brush attached to a copper wire and saturated with the solvent. Continue the distillation for 5 min, then remove the source of heat, and allow the receiving tube to cool to room temperature. If any droplets of water are seen adhering to the wall of the receiving tube, scrub down with a brush consisting of a rubber hand wrapped around a copper wire and dipped with the solvent. When the water and solvent have separated, read the volume of water.

C.5.4 Calculation

$$\text{Water content, percent by mass} = \frac{V \times d \times 100}{m}$$

where

V = Volume of water, in ml, at room temperature collected in the receiving tube;
d = density of water, at room temperature ; and
m = mass, in grams, of the test portion.

C.6 PEROXIDE VALUE

C.6.1 Principle

C.6.1.1 The peroxide value is the quantity of those substances in the sample, expressed in milliequivalents of active oxygen per kilogram of oil, which oxidise potassium iodide under the conditions described.

C.6.1.2 The peroxide value is determined volumetrically by the reaction of potassium iodide in acid solution with the bound oxygen, followed by titration of the liberated iodine with sodium thiosulphate.

C.6.2 Reagents

All reagents and distilled water shall be free from dissolved oxygen.

C.6.2.1 Chloroform, freed from oxygen by flushing with a current of pure, dry inert gas.

C.6.2.2 Glacial acetic acid, freed from oxygen by flushing with a current of pure, dry insert gas.

C.6.2.3 Aqueous potassium iodide solution, fresh, saturated, and free from free iodine and iodates.

NOTE:- Make sure the solution remains saturated as indicated by the presence of undissolved crystals. Store in the dark. Test daily by adding 2 drops of starch solution (C.6.2.5) to 0.5 ml of potassium iodide solution in 30 ml of acetic acids-chloroform (3 volumes: 2 volumes) solution. If a blue colour is formed which requires more than 1 drop of 0.01 N sodium thiosulphate solution to discharge, discard the iodide solution and prepare a fresh solution.

C.6.2.4 Sodium thiosukphate solution 0.002 N or 0.01 N, Standardized just before use.

C.6.2.5 Starch solution, mix 5 g of soluble starch in 30 ml of water, add this mixture to 1 000 ml boiling water and leave boiling for 3 min.

C.6.3 Apparatus

All equipment used shall be free from reducing or oxidising substances.

Note :- Do not grease ground glass surfaces.

C.6.3.1 Flasks, of about 250 ml capacity, with ground necks and ground glass stoppers, dried before hand and filled with a pure, dry inert gas (nitrogen or preferably, carbon dioxide).

C.6.4 Procedure

C.6.4.1 Ensure that the sample is taken and stored away from strong sunlight, kept cold and contained in completely filled glass containers, hermetically sealed with ground glass or cork stoppers.

C.6.4.2 Weigh a portion of the hair cream in a clean, dry beaker such that the weighed portion contains approximately 5 g of oil (see C.4). Wam in a water bath or hot plate till an aqueous layer separates out at the bottom of the beaker. Pour the contents into a centrifuge tube and centrifuge till the separation of a clear upper oil layer occurs if the oil does not separate after centrifuging, discard the bottom aqueous layer and extract the top layer with petroleum ether in a separating funnel. Evaporate off the ether in a water bath from the top layer to obtain the oil. If the eher layer is in the form of an emulsion, transfer into a centrifuge tube and centrifuge till a clear upper oil layer is obtained.

C.6.4.3 Weigh to the nearest milligram, between 2 g and 5 g of the oil obtained as in C.6.4.2 (or less if the sample is clearly rancid) into a 250 ml dry, stoppered counical flask (see C.6.3.1). Add 10 ml of chloroform, dissolve the oil by swirling, add 15 ml of glacial acetic acid and 1 ml fresh saturated aqueous potassium iodide solution. Immediately stopper the flask, shake for 1 min and place the flask for exactly 5 min away from light in a cool, dark place. Add about 75 ml of waer, stir vigorously and titrate the liberated iodine in the presence of a few drops of starch solution with 0.002 N sodium thisulphate solution for expected peroxide values less then or equal to 12 or with the 0.01 N solution for expected peroxide values greater then 12. Carry out reagent blank determination which should not exceed 0.1 ml of 0.01 N thiosulphate solution.

C.6.4.4 Calculation

$$\text{Peroxide value, milliequivalents/kg (see C.6.1.1)} = \frac{(V-V_o)\ N \times 10^3}{m}$$

Where

V = Volume, in ml, of sodium thiosulphate solution used for titration;

Vo = Volume, in ml, of the reagent blank determination;
N = normality of the thiosulphate solution used; and
M = mass, in g, of oil used for the determination.

C.7 MICROBIOLOGICAL EXAMINATION

C.7.1 Apparatus

C.7.1.1 Forceps, scissors, spatula and scalpel, sterile instruments for preparation of sample.

C.7.1.2 Gause pads, sterile, 100 mm x 100 mm.

C.7.1.3 Balance, with weights sensitivity of 0.01 g

C.7.1.4 Mechanical blender, with glass or metal jars fitted with lids and resistant to the conditions of sterilization.

C.7.1.5 Water bath, controlled at 45 ± 2 ^{o}C

C.7.1.6 Incubators, controlled at 36 ± 1 ^{o}C, 30 ± 2 ^{o}C and 42± 1 ^{o}C

C.7.1.7 Petri dishes, sterile, diameter 90 mm or 100 mm

C.7.1.8 Pipettes sterile, graduated, capacity 1, 5 and 10 ml

C.7.1.9 Dilution bottles, wide mouth

C.7.2 Culture media and solutions

Dehydrated media of any brand equivalent to the formulations may be used.

C.7.2.1 Diluent

C.7.2.1.1 Phosphate buffer pH 7.2

C.7.2.1.1.1 Dissolve 34 g of monobasic potassium phosphate in 500 ml of water in a 1 000 ml volumetric flask. Adjust to pH 7.2 by the addition of 1 N NaOH (about 175 ml), add water to volume and mix. Store under refrigeration. For use, dilute the stock solution with water in the ratio f 1:800. Sterilize at 121 ^{o}C for 20 min.

C.7.2.1.2 Modified letheen broth

Prepared from Difco or BBL commercially available medium with the same supplementation as for modified letheen agar (see below) Dispense in appropriate amounts into dilution bottles.

C.7.2.1.3 Modified letheen agar

a)	Letheen agar (Difco or AOAC from BBL)	32 g;
b)	Trypticase peptone	5 g;
c)	Thiotone peptone	10 g;
d)	Yewast extract	2 g;
e)	NaCl	5 g;
f)	Sodium bisulphate	0.1 g; and
g)	Distilled water	1000 ml

Dissolve ingredients boiling.

Autoclave for 15 min at 121 ^{o}C and dispense into 15 mm x 100 mm petri dishes.

C.7.2.2 Plate count agar

a)	Dehydirated yeast extract	2.5 g;
b)	Tryptone	5.0 g;
c)	Anhydrous D-glucose (anhydrous dextrose)	1.0 g;
d)	Agar	9 g to 18 g; and
e)	Distilled water	1000 ml

Dissolve all ingredients in water by b oiling. Sterilize by autoclaving at 121 ^{o}C for 20 min. Final pH 7.0 ± 0.2

C.7.2.3 Trypticase say agar

a)	Trypticase or tryptone	15g;
b)	Phtone	5 g;

c)	NaCl	5 g;
d)	Agar	15 g; and
e)	Water	1000 ml

Suspend and mix thoroughly. Heat with frequent agitation and boil for about 1 min to dissolve completely. Autoclave for 15 min at 121 °C. Final pH 7.3 ± 0.1.

C.7.2.4 Malt extract agar

a)	Maltose	12.75 g;
b)	Dextrose	2.75 g;
c)	Glycerol	2.35 g;
d)	Peptone	0.78 g;
e)	Agar	15 g; and
f)	Water	1000 ml

Suspend all ingredients and mix until homogenous. Heat with frequent agitation and boil for 1 min. Dispense and sterilize by autoclaving at 121 °C for 15 min. Malt extract agar should be supplemented with 40 mg/kg of filter sterilized chlortetracycline or acidified to pH 3.5 with sterile 10 percent citric acid or tartaric acid.

C.7.2.5 Vogel-Johnson (VJ) agar.

C.7.2.6 Cetrimide agar.

C.7.2.7 Fluid soybean casein digect medium

a)	USP pancreatic digest of casein	15.0 g
b)	USP soy peptone	5.0 g
c)	Sodium chloride	5.0 g
d)	Lecithin	0.7
e)	Tween 8O	5.0 g
f)	Agar (dried)	15.0 g and
g)	Distilled water	1000 ml

Sterilize at 118 °C to 121 °C for 15 min. Final pH should be 7.3 ± 0.2

C.7.2.8 Motility test medium

a)	Beef extract	3 g;
b)	Peptone	10 g;
c)	Agar	4 g;
d)	NaCl	5 g;
e)	Distilled water	1000 ml

Adjust pH to 7.4 dispense in 8 ml portions in tubes sterilized for 15 min at 121 °C.

C.7.2.9 Cytochrome oxidase reagent

a) N, -N, -N^1, N^1 – tetramethyl-p-phenylene diamine 3.0 g;
b) Distilled water 100 ml

Store in a dark glass bottle at 5 ^oC to 10 ^oC. Storage life is 15 days.

C.7.2.10 Brain heart infusion broth or nutrient broth

C.7.2.11 Rabbit coagulase plasma (with EDTA)

C.7.2.12 Tween 80

C.7.2.13 Solution of 14 percent HCl in 80 percent alcohol (aqueous).

C.7.3 Procedure

C.7.3.1 Preparation of test sample

C.7.3.1.1 Analyse the samples as soon as possible after arrival in the laboratory. If storage is necessary , the samples should be stored at room temperature.

C.7.3.1.2 Disinfect the surface of the sample container with an aqueous mixture of 80 percent alcohol (v/v) and 1 percent HCl (v/v) Dry the surface with sterile gauze before opening and removing contents.

C.7.3.1.3 Transfer by means of a spataula, using aseptic techniques, 10 g of the product into a widemouth dilution bottle containing 10 ml of Tween 80 and disperse the product withing the Tween 80 with a spatula. Bring volume upto 100 l with either diluent given in C.7.2.1 to obtain a 1 : 10 dilution.

C.7.3.1.4 Prepare decimal dilution from 10^{-1} to 10^{-4} by adding 1 ml of a previous dilution to 10 ml of diluent. Shake all dilutions by rotating tube or with a mechanical shaker.

C.7.3.1.5 Using a sterile pipette transfer 1 ml of each dilution into each of duplicate petri dishes.

C.7.4 Aerobic plate count

C.7.4.1 Pour about 15 ml of plate count agar or ISA previously cooled to 45 ^oC into each petri dish. (see C.7.3.1.5) Rotate the plates carefully to mix the inoculum with the medium. Allow to solidify.

C.7.4.2 Invert the dishes and incubate at 36 ± 1 ^oC in 48 hours.

C.7.4.3 Count colonies on plates containing 30 to 300 colonies and record the results per dilution counted.

If plates do not contain 30 to 300 colonies, record the dilution counted and note the number of colonies found.

C.7.4.4 Average the counts obtained and multiply by the appropriate dilution factor to obtain the number of bacteria/g of sample.

C.7.5 Mould and yeast count

C.7.5.1 Pour about 15 ml of malt extract agar into a second series of Petri dishes countaining 1 ml aliquots of the 10^{-1} to 10^{-4} dilutions.

C.7.5.2 Mix, allow to solidify and incubate plates at 25 ± 1 ^{o}C for 5 days. Check plates daily for growth.

C.7.5.3 Average the counts of duplicate plates containing 30 to 300 colonies, and multiply by dilution factor to obtain the number of fungi and yeast/g sample.

C.7.5.4 The total number of micro organisms per gram is the sum of bacteria, fungi and yeast per gram of sample.

C.7.6 Detection of S. Aureus and P. aeruginosa

C.7.6.1 Enrichment culture

Incubate all dilution bottles prepared as in C.7.3.1.3 and C.7.3.1.4 at 30 ± 2 ^{o}C for a minimum of 7 days, examining the growths daily for growth. If growth is suspected, as well as at the 7 day incubation period, subculture all bottles on to VJ and cetrimide agar plates. Incubate plates at 30 ± 2 ^{o}C for 24 hours. Prepare a gram stain of all morphologrically dissimilar colonial types. If characteristic colonies develop which have the gram staining reactions of S. aureus and P. aeruginosa (see Table 4), the preparation has a presumptive content of these organisms.

TABLE 4 Presumptive tests

Medium (1)	S.aureus (2)	P.aeruginosa (3)
Vogel-Johnson agar	Convex, shiny black colonies with or without yellw zone surrounding the colony	--
Cetrimide agar	--	Generally greenish fluorescence

C.7.6.2 Confirmation of S.aureus

With the aid of an inoculating loop, transfer each suspect colony from the agar surfaces of the VJ medium to individual tubes each containing 0.2 ml brain heart infusion broth or nutrient broth. Incubate for 18 to 24 h at 35 ± 2 ^{o}C, then add 0.5 ml reconstituted rabbit

coagulase plasma (with EDTA) and mix thoroughly. Incubate at 35 ± 2 ^oC, examining at every 3 hour interval upto 24 hours. If coagulation in any degree is observed, Staphylococcus aureus is present.

C.7.6.3 Confirmation of P. aeruginosa

C.7.6.3.1 Perform cytochrome oxidase test on presumptive Pseudomonas colonies by placing a few drops of the cytochrome oxide reagent (see C.7.2.9) on suspect colonies. If the organisms produce oxidase, colonies will turn pink, red, and then black. Discard plates after 20 min. On all oxidase positive colonies perform either a microscopic motility test or a stab and incubate motility agar for 24 hours.

C.7.6.3.2 Transfer oxidase and motility positive colonies to soybean casein digest medium and incubate at 42 ^oC for 24 h to 48 h. Growth at 42 ^oC indicates the presence of Pseudomonas aeruginosa.

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