AMENDMENT NO. 1 APPROVED ON 1985-11-20.

SLS 611 : 1983 SPECIFICATION FOR HAIR CREAMS

Page 16

Clause C.7 - MICROBIOLOGICAL EXAMINATION

Substitute the existing clause with the following:

C.7.1 ***Apparatus***

C.7.1.1 Sterile instruments for preparation of sample – forceps, scissors, spatula and scalpel.

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

C.7.1.3 Balance with weights, sensitivity of 0.01 g.

C.7.1.4 Water baths controlled at 45 ± 2 ^{o}C and 42 ± 1 ^{o}C.

C.7.1.5 Incubators controlled at 36 ± 1 ^{o}C.

C.7.1.6 Petridishes, sterile, 15 mm x 100 mm.

C.7.1.7 Pipettes sterile, graduated, capacity 1 ml, 5 ml and 10 ml.

C.7.1.8 Wide mouth dilution bottles.

C.7.1.9 Glass spreaders, sterile.

C.7.2 ***Culture media and reagents***

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

C.7.2.1 Diluent

C.7.2.1.1 0.1 percent peptone-water diluent

Peptone	:	1.0 g
Distilled water	:	000 ml

Dissolve the ingredients in distilled water. Sterilize at 121 ^{o}C for 15 minutes. Final pH 7.3 ± 0.2.

C.7.2.1.2 Modified letheen broth

Tryptone	10.0 g
Beef extract	5.0 g
Lecithin	0.7 g
Tween 80	5.0 g
Trypticase peptone	5.0 g
Thiotone peptone	10.0 g
Yeast extract	2.0 g
Sodium chloride	5.0 g
Sodium bisulfate	0.1 g
Distilled water	1 000 ml

Dissolve ingredients by boiling. Autoclave for 15 minutes at 121 ^{o}C. Final pH 7.0 ± 0.2. Dispense in appropriate amounts into dilution bottles.

C.7.2.2 Plate count agar (PCA)

Dehydrated yeast extract	2.5 g
Tryptone	5.0 g
Anhydrous D-glucose (anhydrous dextrose)	1.0 g
Agar	9 g to 18 g
Distilled water	1 000 ml

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

C.7.2.3 Trypticase soy agar (ISA)

Trypticase or tryptone	15 g
Phytone	5 g
Sodium chloride	5 g
Agar	15 g
Distilled water	1 000 ml

Suspend and mix thoroughly. Heat with frequent agitation and boil for about 1 minute to dissolve completely. Autoclave for 15 minutes at 15 minutes at 121 ^{o}C. Final pH 7.3 ± 0.1.

C.7.2.4 Vogel - Johnson (VJ) agar

Tryptone	10.0 g
Yeast extract	5.0 g
Mannitol	10.0 g
Dipotassium phosphate	5.0 g
Lithium chloride	5.0 g
Glycine	10.0 g
Phenol red	0.025 g
Agar	15.0 g
Distilled water	1 000 ml

Suspend ingredients in water and heat to boiling to dissolve completely. Sterilize by autoclaving at 121 ^{o}C for 15 minutes. Cool to 45 ^{o}C to 50 ^{o}C and add 20 ml of 1.0 per cent tellurite solution. Final pH 7.2 ± 0.2. Mix thoroughly and dispense in petridishes. Plates may be stored at 4 ^{o}C for upto 7 days.

C.7.2.5 Centrimide agar

Peptone	20.0 g
Magnesium chloride	1.4 g
Potassium sulfate	10.0 g
Centrimide (cetyl trimethyly ammonium bromide	0.3 g
Agar	13.6 g
Distilled water	1 000 ml

Suspend ingredients in water. Add 10 ml of glycerol and heat to boiling to dissolve. Sterilize by autoclaving at 121 ^{o}C for 15 minutes. Final pH 7.2 ± 0.2.

C.7.2.6 Fluid - Soybean casein digest medium (SDC)

USP pancreatic digest of casein (Trypticase)	15.0 g
USP soy peptone	5.0 g
Sodium chloride	5.0 g
Lecithin	0.7 g
Tween 80	5.0 g
Distilled water	1 000 ml

Suspend ingredients in distilled water and dissolve by boiling. Sterilize by autoclaving at 118 ^{o}C to 121 ^{o}C for 15 minutes. Final pH should be 7.3 ± 2.0.

C.7.2.7 Soybean casein digest (SDC) agar

Composition and preparation as for fluid – soybean casein digest medium (.7.2.6), with the addition of 15.0 g of agar per lllitre.

C.7.2.8 Motility test medium

Beef extract	3.0 g
Peptone	10.0 g
Sodium chloride	5.0 g
Agar	4.0 g
Distilled water	1 000 ml

Suspend the ingredients in distilled water and boil for one to two minutes to dissolve. Dispense 1 g 8 ml portions into test tubes. Sterilize by autoclaving at 121 ^{o}C for 15 minutes. Final pH 7.4.

C.7.2.9 Oxidase reagent

Tetramethyl-p-phenylene diamine dihydrochloride	1.0 g
Distilled water	100 ml

Store in dark glass bottles at 5 ^{o}C to 10 ^{o}C.

Storage life is 15 days.

C.7.2.10 Nutrient broth

Beef extract	3.0 g
Peptone	5.0 g
Distilled water	1 000 ml

Suspend ingredients in distilled water and boil to dissolve. Dispense as required and autoclave at 121 ^{o}C for 15 minutes. Final pH 6.8

C.7.2.11 Coagulase plasma with EDIA

Use a commercially available dehydrated rabbit plasma and rehydrate in accordance with the manufacturer's instructions.

C.7.2.12 Tween 80.

C.7.2.13 Aqueous mixture of 80 per cent ethanol (V/V) and 1 per cent hydrochloric acid (V/V).

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 the aqueous mixture (C.7.2.13). Dry the surface with sterile gauze before opening and removing contents.

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

C.7.3.1.4 Shake the dilution bottle 50 items through a distance of 300 mm within 1 minute.

C.7.3.1.5 Prepare serial dilution to obtain a dilution series from 10^{-1} to 10^{-4} by adding 1 ml of previous dilution to 9 ml of either diluent given in C.7.2.1.

C.7.3.1.6 Shake all dilutions by rotating tubes or with a mechanical shaker.

C.7.4 Enumeration of aerobic plate count

C.7.4.1 Using a sterile pipette transfer 1 ml of each dilution into each of duplicate petri dishes.

C.7.4.2 Add about 15 ml of plate count agar (C.7.2.2). Trypticase soy agar (C.7.2.3), or soybean casein digest agar (C.7.2.7) at 45 ^{o}C into each petri dish (C.7.4.1) and rotate the dish carefully to mix the inoculum with the medium. Allow to solidify.

C.7.4.3 Invert the dishes and incubate at 36 ± 1 ^{o}C for 48 hours.

C.7.4.4 Count colonies on plates containing 30 to 300 colonies, and record 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.7.4.5 Average the counts obtained, multiply by the appropriate dilution factor and report the results as the aerobic plate count as per gram of sample.

C.7.5 Detection of *Stophylococous aureus*

C.7.5.1 Transfer 0.5 ml portions from the dilutions prepared in C.7.3.1.5 to duplicate plates of VJ agar (C.7.2.4).

C.7.5.2 Distribute the inoculum over the surface of the agar with a sterile glass spreader.

C.7.5.3 When inoculum has been completely absorbed, invert the plates and incubate at 36 ± 1 ^{o}C for 48 hours.

C.7.5.4 Count plates at the dilution having 30 to 300 well distributed colonies that are convex, shining black, with or without a yellow zone surrounding the colony.

C.7.5.5 Plates having more than 300 colonies may be selected when plates at a greater dilution do not contain the colonial types described above.

Plates from minimal dilutions having less than 30 colonies may also be used if necessary.

C.7.5.6 *Coagulase test*

From each plate demonstrating growth, pick one or more of the typical colonies into tubes containing 0.2 ml nutrient broth (C.7.2.10).

Incubate at 36 ± 1 ^{o}C for 18 hours to 24 hours. Add 0.5 ml of reconstituted rabbit coagulase plasma with EDTA (C.7.2.11), and mix thoroughly.

Incubate at 36 ± 1 ^{o}C and examine for clotting at 3-hour intervals for upto 24 hours.

A known coagulase positive and coagulase negative organism should be included with every set of samples.

C.7.5.7 All strains that yield positive coagulase reactions may be considered a Staphylococous aureus.

C.7.5.8 Calculate the number of Stophylococous aureus organisms present by determining the fraction of colonies tested that are coagulase positive. Multiply this fraction by the average number of characteristic colonies appearing on the VJ agar plates. Multiply the number obtained by the appropriate dilution factor and report as the number of Staphylococous aureus per gram of sample.

C.7.6 Detection of Paeudomonas Geruginese

C.7.6.1 Select different type colonies from the agar plates used in C.7.4..4 and streak plates of Centrimide agar (C.7.7.5).

C.7.6.2 Incubate at 36 ± ^{o}C for 24 hours.

C.7.6.3 Select colonies which show a greenish fluorescence as presumptive Pauedomonas aeruginosa.

C.7.6.4 *Cytechrome oxidase test*

Add a loopful of oxidase reagent (C.7.2.9) to a filter paper in a petri dish. Using a loop, smear the presumptive P, aeruginosa colonies across the moistened paper. A purple colour appearing across the streak within 10 seconds indicates a positive reaction.

C.7.6.5 *Motility test*

Stab motility medium (C.7.2.8) with oxidase positive colonies and incubate at 36 ± ^{o}C for 24 hours. Growth from the line of the stab constitutes a positive test.

C.7.6.6 *Growth at 42 ^{o}C*

Transfer oxidase positive and motility positive colonies to fluid – soybean casein digest medium (C.7.2.6) and incubate at 42 ^{o}C for 24 to 48 hours.

C.7.6.7 Oxidase positive, motility positive colonies which show growth at 42 ^{o}C may be considered a P, *aeruginosa.* Lack of characteristic pigmentation and failure to grow at 42 ^{o}C indicates other *Paeudomonas*.

C.7.6.8 Count the typical colonies on agar plate (C.7.4.4) corresponding to the P, *aeruginosa* colonies selected from that medium and plated on centrimide agar,

C.7.6.9 Multiply by the appropriate dilution factor and report as the number of P, *aeruginosa* per gram of sample.