



**DEAS 339: 2012**

ICS nn.nnn.nn

## **DRAFT EAST AFRICAN STANDARD**

---

**Hair creams, lotions and gels — Specification**

**EAST AFRICAN COMMUNITY**

---

### Copyright notice

This EAC document is copyright-protected by EAC. While the reproduction of this document by participants in the EAC standards development process is permitted without prior permission from EAC, neither this document nor any extract from it may be reproduced, stored or transmitted in any form for any other purpose without prior written permission from EAC.

Requests for permission to reproduce this document for the purpose of selling it should be addressed as shown below or to EAC's member body in the country of the requester:

© East African Community 2012 — All rights reserved  
East African Community  
P.O.Box 1096  
Arusha  
Tanzania  
Tel: 255 27 2504253/8  
Fax: 255 27 2504481/2504255  
E-mail: [eac@eachq.org](mailto:eac@eachq.org)  
Web: [www.eac-quality.net](http://www.eac-quality.net)

Reproduction for sales purposes may be subject to royalty payments or a licensing agreement. Violators may be persecuted

# Contents

Page

Foreword .....	vi
1 Scope .....	1
2 Normative references .....	1
3 Description .....	1
4 Requirements .....	1
4.1 General requirements .....	1
4.2 Specific requirements .....	2
5 Packaging .....	3
6 Marking and labelling .....	3
7 Sampling .....	3
Annex A (normative) Determination of pH .....	4
A.1 Apparatus .....	4
A.2 Procedure .....	4
A.2.1 For gels and oil-in-water emulsion creams or lotions .....	4
A.2.2 For water-in-oil emulsion creams and lotions .....	4
Annex B (normative) Determination of thermal stability .....	5
B.1 Apparatus .....	5
B.2 Procedure .....	5
B.3 Results .....	5
Annex C (normative) Test for rancidity .....	6
C.1 Reagent .....	6
C.2 Procedure .....	6
Annex D (normative) Determination of lead content by graphite furnace Atomic Absorption spectrophotometer (AAS) .....	7
D.1 Scope .....	7
D.2 Warning and safety .....	7
D.3 Principle .....	7
D.4 Materials .....	7
D.4.1 Reagents, chemicals and standards .....	7
D.4.2 Apparatus and equipment .....	8
D.5 Performance .....	8
D.5.1 Sample preparation .....	8
D.5.2 Calibration .....	8
D.6 Procedure .....	9
D.6.1 Test portion .....	9
D.6.2 Blank test .....	9
D.6.3 Instrumentation .....	9
D.6.4 Instrument conditions .....	9
D.7 Expression of results .....	10
D.7.1 Method of calculation .....	10
D.7.2 Expression of results .....	10
D.8 Method validation .....	10
D.8.1 Method validation data .....	10
D.8.2 Precision: Repeatability .....	10
D.8.3 Working range .....	10
D.8.4 Reporting limits .....	11

<b>Annex E (normative) Test for arsenic using Atomic Absorption Spectrophotometer (AAS)</b> .....	12
E.1 Scope .....	12
E.2 Reagents .....	12
E.3 Stock solution .....	12
E.4 Calibration solution .....	12
E.4.4 Calibration volume: .....	12
E.5 Reductant solution .....	12
E.6 Oxidation state .....	13
E.7 Prereduction .....	13
E.7.1 KI solution .....	13
E.7.2 L-cysteine solution .....	13
E.8 Instrument conditions .....	13
E.9 Notes .....	14
<b>Annex F (normative) Test for mercury using Atomic Absorption Spectrophotometer (AAS)</b> .....	15
F.1 Method 1: .....	15
F.1.1 Scope .....	15
F.1.2 Reagents .....	15
F.1.3 Stock solution .....	15
F.1.4 Calibration solution .....	15
F.1.5 Reductant solution .....	16
F.1.6 Instrument conditions .....	16
F.1.7 Notes .....	16
F.2 Using tin (II) chloride (SnCl <sub>2</sub> ) as reductant .....	16
F.2.1 Scope .....	16
F.2.2 Reagents .....	16
F.2.3 Calibration solution .....	17
F.2.4 Reductant solution .....	17
F.2.5 Instrument conditions .....	17
F.2.6 Notes .....	17
<b>Annex G (normative) Microbiological examination</b> .....	18
G.1 Outline of the method .....	18
G.2 Apparatus .....	18
G.2.1 Tubes .....	18
G.2.2 Autoclaves .....	18
G.2.3 Petri dishes .....	18
G.2.4 Colony counter .....	18
G.3 Media and buffer .....	18
G.3.1 Soyabean casein digest agar media .....	18
G.3.2 Stock solution ph phosphate buffer .....	19
G.3.3 Dilute phosphate buffer solution pH 7.2 .....	19
G.4 Sterilization of apparatus .....	19
G.4.1 Tubes .....	19
G.4.2 Petri-dishes .....	19
G.4.3 Pipettes .....	19
G.5 Procedure .....	19
G.6 Expression of results .....	19
<b>Annex H (normative) Determination of <i>pseudomonas aeruginosa</i>, <i>staphylococcus aureus</i> and <i>candida albicans</i> in cosmetic products</b> .....	20
H.1 Introduction .....	20
H.2 Principle of the method .....	20
H.3 Apparatus .....	20
H.4 Reagents .....	21
H.4.1 Lethen agar .....	21
H.4.2 D/E Neutralizing broth (Dey/Engley) .....	21
H.4.3 Nutrient agar .....	21
H.4.4 Y/M agar (yeast/malt extract) .....	21
H.4.5 Potato Dextrose Agar (PDA) .....	22
H.4.6 0.85 % saline .....	22

H.4.7	0.85 % saline with 0.05 % polysorbate 80 .....	22
H.4.8	Barium sulphate standard No 2 .....	22
H.4.9	Barium sulphate standard No. 7 .....	22
H.5	Microorganisms .....	22
H.6	Product quality check .....	23
H.7	Product preparation .....	23
H.8	Bacterial inocula preparation.....	23
H.9	Fungal inoculum preparation.....	23
H.10	Inoculum pools .....	24
H.11	Inoculation .....	24
H.12	Sampling intervals.....	24
H.13	Aerobic Plate Count (APC) .....	24
H.14	Neutralization check.....	25
H.15	Data analysis.....	25
	Annex I (normative) Total heavy metals.....	26
	Bibliography.....	27

## **Foreword**

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in the East African Community. It is envisaged that through harmonized standardization, trade barriers that are encountered when goods and services are exchanged within the Community will be removed.

In order to achieve this objective, the Community established an East African Standards Committee mandated to develop and issue East African Standards.

The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the procedures of the Community.

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

PUBLIC REVIEW

## Hair creams, lotions and gels — Specification

### 1 Scope

This East African Standard specifies the requirements and methods of test for hair creams, lotions and gels based on vegetable oil or mineral oil, or any combination of the above, with fatty acids or fatty acid emulsions.

It applies to hair conditioners and setting lotions.

This standard does not cover hair sprays, hair sheens or hair oils including hair creams, lotions and gels for which therapeutic claims are made.

### 2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

*EAS 334*, Illustrative list by category of cosmetic products

*DEAS 346* Labelling of cosmetics — General requirements

*DEAS 377-2*, Cosmetics — List of substances which must not form part of the composition of any cosmetic product.

*DEAS 377-3*, Cosmetics — List of substances which cosmetics must not contain except subject to the restrictions and conditions laid down.

EAS 381

### 3 Description

Hair creams, lotions and gels are products used to

- a) enhance glossy appearance on the hair; stimulate the scalp and used as an anti-dandruff; and condition the hair.

### 4 Requirements

#### 4.1 General requirements

**4.1.1** The hair cream and lotion shall be in the form of an emulsion and be of a uniform colour showing no signs of separation nor visible impurities.

**4.1.2** Materials used, including dyes, colours, pigments, perfumes or preservatives if added, shall be of pharmaceutical or cosmetic grade and shall comply with DEAS 377-3.

**4.1.3** Unjustifiable claims are prohibited by this standard. Additives, when used shall be of acceptable amounts to effect the intended end performance as stipulated on the label e.g. conditioning.

**4.1.4** The product shall not be harmful to the scalp or hair when used as intended by the manufacturer.

**4.1.5** Any product containing ingredients for which medicinal claims are made shall be registered with the Ministry responsible for Health matters.

## 4.2 Specific requirements

The hair cream lotion or gel shall comply with the pH requirements as specified in Table 1.

**Table 1 — pH requirements for hair creams, lotions and gels**

SI No	Product	pH requirement	Test method
i)	Hair cream gel or lotion	5- 8	Annex A
ii)	Hair conditioners	3- 6	Annex A
a Hair conditioners shall include lotions as well as cream treatment conditioners (rinse off).			

The hair cream lotion or gel shall comply with other requirements as specified in Table 2 when tested according to the specified methods of test.

**Table 2: requirements for hair creams, lotions and gels**

Characteristic	Requirements	Test method
i)	Thermal stability	To pass test
ii)	Rancidity	To pass test
vi)	Microbiological examination Total viable count for aerobic mesophyllic micro-organisms per g, max	100
vii)	<i>Pseudomonas aeruginosa</i> <i>staphylococcus aureus</i> <i>Candida albicans</i>	Not detectable in 0.5g of cosmetic product
viii)	Total amount of heavy metals	20 ppm

The products shall comply with the requirements for contaminants in accordance with Table 3.

**Table 3: Requirements for contaminants**

S/N	Characteristic	Requirement	Method of test
1	Lead, ppm, max	20	Annex C
2	Arsenic, ppm, max	2	Annex D
3	Mercury, ppm, max	2	Annex E
NOTE The total amount of heavy metals as lead, mercury and arsenic, in combination, in the finished product should not exceed 20 ppm.			



## 5 Packaging

The product shall be packed in suitable well-sealed containers that shall protect the contents and shall not cause any contamination or react with the product.

## 6 Marking and labelling

The containers shall be securely closed and in addition to the labelling requirements of DEAS 346. The labelling shall be in either English, Kiswahili or French or in combination as agreed between the manufacturer and supplier. The following information shall be indelibly and legibly marked on the container:

- a) product name;
- b) net contents;
- c) Manufacturer's name, physical address, and trade mark (if any) and name and physical address of the distributor/supplier if any
- d) batch number in code or otherwise;
- e) the date of manufacture in the form "mm/yyyy",
- f) best before date in the form "mm/yyyy"; and
- g) Country of origin
- h) instructions for use;
- i) all ingredients shall be declared in descending order of predominance. The INCI label names shall be used.

NOTE INCI stands for International Nomenclature Cosmetic Ingredient.

## 7 Sampling

Representative samples shall be drawn for test from the market or anywhere else following the procedure outlined in EAS 381. The samples shall be declared as complying with the specification if they satisfy all the specified requirements.

## **Annex A** (normative)

### **Determination of pH**

#### **A.1 Apparatus**

A pH meter preferably equipped with a glass electrode.

#### **A.2 Procedure**

##### **A.2.1 For gels and oil-in-water emulsion creams or lotions**

Weigh  $5 \text{ g} \pm 0.01 \text{ g}$  of the sample in a 100-mL beaker. Add 45 mL of water and disperse the sample in it. Determine the pH of the suspension at  $25^\circ \text{ C}$  using the pH meter.

##### **A.2.2 For water-in-oil emulsion creams and lotions**

Weigh 10 g of the cream to the nearest 0.1 g. Add 90 mL of rectified spirit previously adjusted to pH 6.5 to 7.0. Warm, if necessary, to  $45^\circ \text{ C}$  and stir thoroughly for 15 min. Filter the alcohol layer through a filter paper and measure the pH of the filtrate at  $25^\circ \text{ C}$  using pH using meter.

NOTE Determine the form of cream by placing some of it on spot tile and sprinkling with a mixed indicator consisting of an intimate mixture of oil soluble dye of one colour; e.g. oil orange, and a water-soluble dye of a different colour e.g. methylene blue. After a few minutes, the predominant colour indicates whether the continuous phase is oil or water. In case of doubt, matter is confirmed by checking whether the product is capable of conducting electricity: if so the cream is deemed to be watercontinuous.

## **Annex B** (normative)

### **Determination of thermal stability**

#### **B.1 Apparatus**

A thermostatically controlled oven, capable of maintaining a temperature of  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ .

#### **B.2 Procedure**

Place a fresh unopened sample of the product in its original container into a thermostatically controlled oven at  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for 48 h, making sure that the sample is securely sealed. If the product is packed in an opaque container (e.g. a tube), remove 50 g of the sample and place into an effectively sealed tube or vial, and test as above.

#### **B.3 Results**

The products shall be taken to have passed the test if, on removal from the oven, the following indications of instability are not observed:

- a) change of colour;
- b) Change of smell or odour;
- c) Phase separation;
- d) Formation of granules or crystal growth;
- e) Shrinkage due to evaporation of water.

**Annex C**  
(normative)

**Test for rancidity**

**C.1 Reagent**

**C.1.1** Concentrated hydrochloric acid

**C.1.2** Phloroglucinol solution

Dissolve 0.1g of phloroglucinol in 100 mL of diethyl ether.

**C.2 Procedure**

**C.2.1** Shake 10 mL of the material, melted if necessary, with 10 mL of concentrated hydrochloric acid and 10 mL of phloroglucinol solution. Shake for 1 min.

**C.2.2** The sample shall be taken to have passed the test if no pink colour develops.

## Annex D (normative)

### Determination of lead content by graphite furnace Atomic Absorption spectrophotometer (AAS)

#### D.1 Scope

This test method specifies an electro thermal atomization technique using graphite furnace AAS method for the determination of lead content of foam baths and shower gels.

#### D.2 Warning and safety

The acids used in the test are highly corrosive and should be handled with maximum care and where appropriate, use a fume hood during preparation of standards. Lead is very toxic/carcinogenic and must be handled with maximum care avoiding physical contact.

If spillage occurs, use adequate amounts of water and soap to wash off the spill.

#### D.3 Principle

Injecting of the prepared solution into a graphite furnace. Spectrometric measurements of the atomic absorption of the 228.8 spectral line emitted by lead hollow cathode lamp.

#### D.4 Materials

##### D.4.1 Reagents, chemicals and standards

###### D.4.1.1 Nitric acid,

$\rho$  about 1.4 g/mL.

###### D.4.1.2 Nitric acid (1+1) v/v

Mix 1 volume of conc. HNO<sub>3</sub> with 1 volume of distilled water.

###### D.4.1.3 Nitric acid (0.1M)

Place 17 mL of concentrated acid in 100-mL volumetric flask, then top to the mark with distilled water and mix.

###### D.4.1.4 Lead standard solution

1 000 ppm, in 1-litre volumetric flask, dissolve 1.598 g of Pb(NO<sub>3</sub>)<sub>2</sub> in minimum volume of 1 % v/v HNO<sub>3</sub> and finally top the mark using 1 % HNO<sub>3</sub>.

NOTE Commercial grade standards can also be used when available.

#### **D.4.1.5 Lead standard solution**

100ppb, this shall be prepared freshly by serial dilution of the lead solution (4.1.4).

#### **D.4.1.6 Purge gas**

Argon, sufficiently pure, free from water and oil and free from lead.

### **D.4.2 Apparatus and equipment**

#### **D.4.2.1 Atomic Absorption Spectrometer fitted with graphite furnace**

The Atomic Absorption Spectrometer used shall be satisfactory if after optimization according to the manufacturers instructions and when in reasonable agreement with the values given by the manufacturer and it meets the performance criteria as set out in the manual.

#### **D.4.2.2 Lead hollow cathode lamp**

#### **D.4.2.3 Ordinary laboratory apparatus**

NOTE All glassware shall first be washed in hydrochloric acid ( $\rho$  about 1.19 g/mL, diluted.)

### **D.5 Performance**

#### **D.5.1 Sample preparation**

Ignite 1 g of sample at  $500 \pm 2 \text{ }^\circ\text{C}$  to ash. Extract the lead from the ash with 20 mL of 2 N  $\text{HNO}_3$ , and repeat with 10 mL of 2 N  $\text{HNO}_3$ . Combine the extracts and dilute to 50 mL with 0.5 N  $\text{HNO}_3$ .

#### **D.5.2 Calibration**

##### **D.5.2.1 Preparation of calibration curve**

**D.5.2.1.1** Dilute the stock 100 ppb solution with 0.1M  $\text{HNO}_3$  to obtain solutions with 10 ppb, 20 ppb, 40 ppb, 60 ppb, 80 ppb and 90 ppb of lead.

**D.5.2.1.2** Inject 20 microlitres each of the six solutions in turns at the same rate starting from the lowest concentrated solution to the highest concentrated solution.

**D.5.2.1.3** Record the corresponding absorbance values and plot calibration curve.

##### **D.5.3 Quality control checks**

###### **D.5.3.1 Duplicates**

**D.5.3.1.1** All samples will be analyzed in duplicates and the stated acceptance criteria shall apply. The absolute difference between two independent test results obtained using the same procedure shall not be greater than 10 % of the arithmetic mean of the two results.

**D.5.3.1.2** Spike distilled water with 10.0 ppb of lead and obtain the recovery percentage.

**D.5.3.1.3** Recovery %  $\geq 96$ .

## D.6 Procedure

### D.6.1 Test portion

Use sample as prepared in (D.5.1).

### D.6.2 Blank test

D.6.2.1 Run a parallel reagent blank determination replacing the test solution with distilled water.

D.6.2.2 Reagent blank should be  $\leq 0.0001$  ppb of lead.

### D.6.3 Instrumentation

D.6.3.1 Follow the manufacturer's instructions for preparing the instrument for use.

D.6.3.2 Install the appropriate lamp and adjust the current to the recommended value.

D.6.3.3 Ensure that the autosampler pipette is correctly aligned and that the drain is available.

D.6.3.4 Select the sample tray type installed.

D.6.3.5 Ensure that the graphite tube is in good condition and correctly aligned.

D.6.3.6 Switch on the cooling system, turn on the purge gas and finally start the instrument software.

D.6.3.7 Select the relevant method and then condition tube.

### D.6.4 Instrument conditions

The following conditions shall be used for the furnace during analysis of lead:

D.6.4.1 Wavelength: 283.3

D.6.4.2 Slit: 0.7

D.6.4.3 Atomization site: pyro/platform

**Table D.1 — Furnace conditions for lead**

Step	Temperature °C	Ramp (seconds)	time	Hold (seconds)	time	Internal gas flow (L/ min)	Gas type
1 (drying)	120	10		60		250	Normal
2 (pretreatment)	700	1		30		250	Normal
3 (cooling)	20	1		15		250	Normal
4 (atomization)	1800	0		5		0	Normal
5 (cleanout)	2600	1		5		250	Normal

### D.6.5 Spectrometric measurements

D.6.5.1 Inject into the flame the calibration standards, the blank solution and the test solution.

D.6.5.2 Record the absorbance reading.

**D.6.5.3** If the absorbance of the sample is greater than the highest calibration standard, dilute the test solution appropriately using 0.1 M HNO<sub>3</sub> for lead; then measure the absorbance.

**D.6.5.4** Inject the calibration solutions in ascending order.

NOTE The calibration curve shall only be acceptable for analysis when the correlation coefficient ( $r \geq 0.99$ ).

## D.7 Expression of results

### D.7.1 Method of calculation

The lead content of the sample expressed in mg/L of product is equal to:

$$[(C1 - C2) * V] / M0$$

where,

C1 = lead content of test solution expressed in mg/L read from calibration curve;

C2 = lead content of blank solution expressed in mg/L read from calibration curve;

M0 = grams of sample taken for analysis (5 g);

V = volume of sample taken for analysis (100 mL).

NOTE If the test solution was diluted, then the dilution factor shall be taken into consideration in calculation.

### D.7.2 Expression of results

Report results of lead content in mg/L as Pb in the sample into two decimal points.

## D.8 Method validation

### D.8.1 Method validation data

**Table 2 — Method validation data**

Element	Linearity	LOQ ppb	LOD ppb
Pb	$r \geq 0.99$	32.356	3.804

### D.8.2 Precision: Repeatability

The absolute difference between two independent tests results obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time should not be greater than 10 % of the arithmetic mean of the two results.

### D.8.3 Working range

Pb 10 - 100 ppb.



#### **D.8.4 Reporting limits**

Pb 30 ppb.

PUBLIC REVIEW

## Annex E (normative)

### Test for arsenic using Atomic Absorption Spectrophotometer (AAS)

#### E.1 Scope

This method describes the determination of arsenic in foam baths and shower gels.

#### E.2 Reagents

**E.2.1** 0.15 mol/L ( $\cong$  1.5 % v/v) hydrochloric acid, carefully add 15 mL conc. HCl to deionized water and make up to 1 L.

**E.2.2** 0.25 mol/L ( $\cong$  1 % w/v) NaOH solution, carefully dissolve 10 g sodium hydroxide flakes in deionized water and make up to 1 L.

**E.2.3** 0.8 mol/L ( $\cong$  3 % w/v) NaBH<sub>4</sub> solution, dissolve 3 g sodium tetrahydroborate in 1 % NaOH solution and make up to 100 mL with 1 % NaOH solution.

#### E.3 Stock solution

The stock solution contains 1 000 mg/L As. The use of commercially available concentrated calibration solutions for AAS is recommended.

WARNING! Arsenic solutions are toxic.

#### E.4 Calibration solution

1 mg As/L (in 1.5 % HCl)

**E.4.1** Aliquots for calibration: 10, 25, 50  $\mu$  L

**E.4.2** Corresponding to: 10, 25, 50 ng As

**E.4.3** Diluent: 1.5 % (v/v) hydrochloric acid

#### E.4.4 Calibration volume:

10 mL

#### E.5 Reductant solution

3 % NaBH<sub>4</sub> in 1 % NaOH solution.

## E.6 Oxidation state

The hydride is generated much more slowly from As (V) than from As (III). To prevent interferences,

As (V) must be prereduced to As (III) prior to the determination. Prereduction can be performed with KI in semi concentrated (5 mol/L) HCl solution or, preferably, with L-cysteine.

## E.7 Prereduction

### E.7.1 KI solution

Dissolve 3 g KI and 5 g L (+) ascorbic acid in 100 mL water. Prepare fresh daily. Add 1 mL of the KI solution per 10 mL of the sample solution in 5 mol/L HCl and stand for 30 min. or,

### E.7.2 L-cysteine solution

Dissolve 5 g L-cysteine in 100 mL 0.5 mol/L HCl. This solution is stable for at least a month. Add 2 mL of the L-cysteine solution per 10 mL of the sample solution and stand for 30 min.

## E.8 Instrument conditions

E.8.1 Analytical wavelength: 193.7 nm

E.8.2 Slit width and height: 0.7 nm Low

E.8.3 Radiation source: Electrodeless discharge lamp for As.

E.8.4 QTA heating: Heat the QTA in a lean, blue air-acetylene flame.

E.8.5 Prepared measurement volume: 10 mL minimum to 50 mL maximum.

E.8.6 Pre-reaction purge time: Approx. 50 s

E.8.7 Post-reaction purge time: Approx. 40 s

E.8.8 Characteristic mass: 0.95 ng As for 1 % absorption ( $A = 0.0044$ ).

E.8.9 Characteristic concentration: 0.095  $\mu$ g/L 1 % absorption for 10 mL calibration volume.

E.8.10 Characteristic concentration check: 50  $\mu$ L of the 1000 mg/L As stock solution (50 ng) give an absorbance of approx.  $A = 0.2$ .

### Alternate analytical wavelengths

Wavelength nm	Slit width nm	Sensitivity relative to main analytical wavelength
189.0	0.7	0.8
197.2	0.7	2.0

## E.9 Notes

Condition the QTA in cold hydrofluoric acid if there is a decrease in sensitivity (and other causes are excluded).

PUBLIC REVIEW

## Annex F (normative)

### Test for mercury using Atomic Absorption Spectrophotometer (AAS)

#### F.1 Method 1:

Using sodium tetrahydroborate ( $\text{NaBH}_4$ ) as reductant

##### F.1.1 Scope

This method describes the determination of mercury in pomades and solid brilliantines using sodium tetrahydroborate ( $\text{NaBH}_4$ ) as reductant

##### F.1.2 Reagents

**F.1.2.1** 0.15 mol/L ( $\cong$  1.5 % v/v) hydrochloric acid, carefully add 15 mL conc. HCl to deionized water and make up to 1 L.

**F.1.2.2** 0.22 mol/L ( $\cong$  1.5 % v/v) nitric acid, carefully add 15 mL conc.  $\text{HNO}_3$  to deionized water and make up to 1L.

**F.1.2.3** 5 % (w/v)  $\text{KMnO}_4$  solution, dissolve 5 g potassium permanganate in deionized water and make up to 100 mL.

**F.1.2.4** 0.25 mol/L ( $\cong$  1 % w/v) NaOH solution, carefully dissolve 10 g sodium hydroxide flakes in deionized water and make up to 1L.

**F.1.2.5** 0.8 mol/L ( $\cong$  3 % w/v)  $\text{NaBH}_4$  solution, dissolve 3 g sodium tetrahydroborate in 1 % NaOH solution and make up to 100 mL with 1 % NaOH solution.

##### F.1.3 Stock solution

The stock solution contains 1 000 mg/L Hg. The use of commercially available concentrated calibration solutions for AAS is recommended.

WARNING! Mercury solutions are toxic.

##### F.1.4 Calibration solution

1 mg Hg/L (in 1.5 %  $\text{HNO}_3$  stabilized by the addition of a few drops of 5 %  $\text{KMnO}_4$  solution)

**F.1.4.1** Aliquots for calibration: 100, 200, 500  $\mu$  L

**F.1.4.2** Corresponding to: 100, 200, 500 ng Hg

**F.1.4.3** Diluent: 1.5 % (v/v) nitric acid or 1.5 % (v/v) hydrochloric acid

**F.1.4.4** Calibration volume: 10 mL

### F.1.5 Reductant solution

3 % NaBH<sub>4</sub> in 1 % NaOH solution

### F.1.6 Instrument conditions

F.1.6.1 Analytical wavelength: 253.6 nm

F.1.6.2 Slit width and height: 0.7 nm Low

F.1.6.3 Radiation source: Electrodeless discharge lamp or hollow cathode lamp for Hg.

F.1.6.4 QTA heating: No flame required. If condensation in the QTA is a problem, heat the QTA gently by mounting an infrared lamp above it.

F.1.6.5 Prepared measurement volume: 10 mL minimum to 50 mL maximum.

F.1.6.6 Pre-reaction purge time: Approx. 5 s

F.1.6.7 Post-reaction purge time: Approx. 50 s

F.1.6.8 Characteristic mass: 4.68 ng Hg for 1 % absorption ( $A = 0.0044$ ).

F.1.6.9 Characteristic concentration: 0.468  $\mu$ g/L 1 % absorption for 10 mL calibration volume.

F.1.6.10 Characteristic concentration check: 250  $\mu$  L of the 1000 mg/L Hg stock solution (250 ng) give an absorbance of approx.  $A = 0.2$ .

### F.1.7 Notes

F.1.7.1 Stabilize stock and calibration solutions by adding KMnO<sub>4</sub> or KI solution.

F.1.7.2 Stabilize all solutions in the reaction flask by adding 1 drop of 5 % (w/v) KMnO<sub>4</sub> solution before starting the determination.

## F.2 Using tin (II) chloride (SnCl<sub>2</sub>) as reductant

### F.2.1 Scope

This method describes the determination of mercury in pomades and solid brilliantines using tin (II) chloride (SnCl<sub>2</sub>) as reductant.

### F.2.2 Reagents

F.2.2.1 0.15 mol/L ( $\cong$  1.5 % v/v) hydrochloric acid, carefully add 15 mL conc. HCl to deionized water and make up to 1 L.

F.2.2.2 1 mol/L ( $\cong$  10 % v/v) hydrochloric acid, carefully add 100 mL conc. HCl to deionized water and make up to 1L.

F.2.2.3 0.22 mol/L ( $\cong$  1.5 % v/v) nitric acid, carefully add 15 mL conc. HNO<sub>3</sub> to deionized water and make up to 1L.

F.2.2.4 5 % (w/v) KMnO<sub>4</sub> solution, dissolve 5 g potassium permanganate in deionized water and make up to 100 mL.

**F.2.2.5** 5 % (w/v) SnCl<sub>2</sub>, dissolve 50 g tin (II) chloride dihydrate (SnCl<sub>2</sub>·2H<sub>2</sub>O) in 10 % HCl solution and make up to 1 L with 10% HCl solution.

#### **F.2.2.6 Stock solution**

The stock solution contains 1 000 mg/L Hg. The use of commercially available concentrated calibration solutions for AAS recommended.

WARNING! Mercury solutions are toxic.

#### **F.2.3 Calibration solution**

1 mg Hg/L (in 1.5 % HNO<sub>3</sub> stabilized by the addition of a few drops of 5 % KMNO<sub>4</sub> solution)

**F.2.3.1** Aliquots for calibration: 100, 200, 500 μ L

**F.2.3.2** Corresponding to: 100, 200, 500 ng Hg

**F.2.3.3** Diluent: 1.5 % (v/v) nitric acid or 1.5 % (v/v) hydrochloric acid

**F.2.3.4** Calibration volume: 10 mL

#### **F.2.4 Reductant solution**

5 % SnCl<sub>2</sub>·O. 2H<sub>2</sub>O in 10 % HCl solution.

#### **F.2.5 Instrument conditions**

**F.2.5.1** Analytical wavelength: 253.6 nm

**F.2.5.2** Slit width and height: 0.7 nm Low

**F.2.5.3** Radiation source: Electrodeless discharge lamp or hollow cathode lamp for Hg

**F.2.5.4** QTA heating: No flame required. If condensation in the QTA is a problem, heat the QTA gently by mounting an infrared lamp above it.

**F.2.5.5** Prepared measurement volume: 10 mL minimum to 50 mL maximum

**F.2.5.6** Pre-reaction purge time: Approx. 5 s

**F.2.5.7** Post-reaction purge time: Approx. 50 s

**F.2.5.8** Characteristic mass: 4.68 ng Hg for 1 % absorption (A = 0.0044)

**F.2.5.9** Characteristic concentration: 0.468 μ g / L / 1 % absorption for 10 mL calibration volume

**F.2.5.10** Characteristic concentration check: 250 μ L of the 1000 mg/L Hg stock solution (250 ng) give an absorbance of approx. A = 0.2

#### **F.2.6 Notes**

**F.2.6.1** Stabilize stock and calibration solutions by adding KMNO<sub>4</sub> solution. Do not use KI solution since iodide interferes in the release of mercury.

**F.2.6.2** Stabilize all solutions in the reaction flask by adding 1 drop of 5 % (w/v) KMnO<sub>4</sub> solution before starting the determination.

## **Annex G** (normative)

### **Microbiological examination**

#### **G.1 Outline of the method**

The test consists of plating a known dilution of the sample or any digest agar medium (soyabean casein is recommended) suitable for the total count of aerobic bacteria and fungi after incubating them for a specified period to permit the development of visual colonies.

IMPORTANT Take precaution in ascertaining that only fresh samples, from carefully sealed containers that had not been opened before, are used for this test. This is very necessary for getting accurate results.

#### **G.2 Apparatus**

##### **G.2.1 Tubes**

Of resistant glass, provided with closely fitting metal caps.

##### **G.2.2 Autoclaves**

Of sufficient size. They shall keep uniform temperature within the chamber up to and including the sterilizing temperature of 122 ° C. They shall be equipped with an accurate thermometer, located so as to register the minimum temperature within the sterilizing chamber, a pressure gauge and, properly adjusted safety valves.

##### **G.2.3 Petri dishes**

Of 100 diameter and 15 mm depth. The bottom of the dishes shall be free from bubbles and scratches and shall be flat so that the medium is of uniform thickness throughout the plate.

##### **G.2.4 Colony counter**

An approved counting aid, such as a Quebec colony counter. If such a counter is not available, counting may be done with a lens giving a magnification of 1.5 diameter. In order to ensure uniformity of conditions during counting, illumination equivalent to that provided by the Quebec colony counter shall be employed.

#### **G.3 Media and buffer**

##### **G.3.1 Soyabean casein digest agar media**

Dissolve 1.5 g of pancreatic digest of casein, 5 g of papic digest of soyabean meal; and 5 g of sodium chloride in 100 mL of distilled water contained in a 2-litre beaker by heating in a water-bath. Add 15 g of powdered agar and continue boiling until the agar is completely digested. Adjust the pH to 7.5 with sodium hydroxide solution. Distribute in 20 mL quantities; close the tubes with metal cups and autoclave at 122 ° C for 20 min. After auto-claving, store the tubes in a cool place and use them within 3 weeks.



### **G.3.2 Stock solution ph phosphate buffer**

Dissolve 34 g of monobasic potassium in about 500 mL of water contained in a 100 mL volumetric flask. Adjust the pH to  $7.2 \pm 0.1$  by the addition of sodium hydroxide solution (4 %). Add water to volume and mix. Sterilise at  $122^{\circ}\text{C}$  for 20 min, store under refrigeration.

### **G.3.3 Dilute phosphate buffer solution pH 7.2**

Dilute 1 mL of stock solution with distilled water in the ratio of 1: 800. Fill 50 mL each in conical flasks of 100-mL capacity. Plug the flasks with cotton and sterilize at  $122^{\circ}\text{C}$  for 20 min.

## **G.4 Sterilization of apparatus**

### **G.4.1 Tubes**

These shall be sterilized in the autoclave at a temperature of  $122^{\circ}\text{C}$  and 1.05 kg/cm pressure for 20 min or in the hot air oven at  $180^{\circ}\text{C}$  for one hour.

### **G.4.2 Petri-dishes**

These shall be packed in drums and autoclaved at  $122^{\circ}\text{C}$  and 1.85 kg/cm pressure for 20 min or individually wrapped in Kraft paper and sterilized in hot oven at  $160^{\circ}\text{C}$  for one hour.

### **G.4.3 Pipettes**

These shall be placed in pipette cones (copper, stainless steel or aluminium) after plugging the broader end with, cotton and sterilized in the autoclave at  $122^{\circ}\text{C}$  and 1.05 kg/cm<sup>3</sup> pressure for 20 min or at  $160^{\circ}\text{C}$  for one hour in hot air oven.

## **G.5 Procedure**

**G.5.1** Melt a sufficient number of soyabean casein digest agar medium tubes in hot water-bath and transfer while hot into a constant temperature water-bath maintained at  $48^{\circ}\text{C} \pm 2^{\circ}\text{C}$ .

**G.5.2** Weigh and transfer aseptically 1 g of the sample to a conical flask containing sterile 50-mL, or any suitable dilution factors, of dilute phosphate buffer at pH 7.2. Shake well. Pipette out in 1-mL portions into three sterile petri dishes. Pour melted and cooled (at  $45^{\circ}\text{C}$ ) soyabean casein digest agar medium over it, and rotate the plates to mix thoroughly. Incubate the plates at  $32^{\circ}\text{C}$  for 72 h in an inverted position.

## **G.6 Expression of results**

Get the average number of colonies on soya-bean casein digest agar medium plates determine the number of micro-organisms per gram of the sample. If no colony is recovered from any of the plates it can be stated as less than 50 micro organisms per gram.

## Annex H (normative)

### Determination of *pseudomonas aeruginosa*, *staphylococcus aureus* and *candida albicans* in cosmetic products

#### H.1 Introduction

This method is obtained from the AOAC Official Method 998.10, *Efficacy of preservation of non-eye area water-miscible cosmetic and toiletry formulations*.

Acknowledge of microbiological techniques is required for these procedures. A general aseptic and safety procedures should be followed. Table H.1 gives the results of the interlaboratory study supporting the acceptance of the method.

#### H.2 Principle of the method

Bacteria yeast and mould grown on laboratory media, harvested, calibrated, and inoculated into test products. Using serial dilutions and plate counts; the numbers of organisms surviving in the test products are determined over time. Products meeting the specified criteria are considered adequately preserved for manufacture and consumer use. Products not meeting criteria are considered inadequately preserved.

**Table H.1 Inter-laboratory study results for determination of preservation of non-eye area water-miscible cosmetic and toiletry formulations**

Incidence of false- negatives among total positive samples A				Incidence of false- positives among total positive samples B		
Product name	Number	Percentage	sensitivity rate	Number	Percentage	sensitivity rate
Shampoo	2/49	4	96	0/53	0	100
Conditioner	3/48	6	94	0/54	0	100
Water-in oil emulsion	0/52	0	100	1/50	2	98
Oil- in- water emulsion	0/51	0	100	0/51	0	100
All combined	5/200	2	98	1/208	0.5	99.5
a) False- negative analysis indicates a sample is adequately preserved.						
b) False- positive analysis indicates a sample is not adequately preserved.						

#### H.3 Apparatus

**H.3.1** Jars 2 to 4 oz wide-mouth, straight-side flint glass ointment jars with linerless metal, polypropylene or teflon lined screw caps.

**H.3.2** Disposable borosilicate glass culture tubes, 16 × 125 mm, with caps.

**H.3.3** Disposable borosilicate glass culture tubes, 20 × 150 mm, with screw caps.

**H.3.4** Petri plates, 100 x 15 mm.

**H.3.5** Sterile 2.2 mL pipettes.

**H.3.6** Sterile swabs.

**H.3.7** Glass beads.

**H.3.8** Sterile gauze.

**H.3.9** 10-20  $\mu$  L inoculating loops.

**H.3.10** Vortex mixer.

## **H.4 Reagents**

For convenience, dehydrated media of any brand equivalent in function may be used. Test each lot of medium for sterility and growth-promotion using suitable organisms.

### **H.4.1 Lethen agar**

Contains 5.0 g pancreatic digest of casein 1.0 g dextrose, 3.0 g beef extract, 1.0 g lecithin, 7.0 g polysorbate 80, and 15.0 g agar per L. Prepare according to manufacturer's directions. Dispense into suitable containers and sterilize by autoclaving at 121 ° C for 15 min. Final pH should be 7.0 ± 0.2 at 25 ° C. Place in 45 ° C water bath until agar is 45 ° C ± 2 ° C. Use for pour plates.

### **H.4.2 D/E Neutralizing broth (Dey/Engley)**

Contains 5.0 g pancreatic digest of casein, 2.5 g yeast extract, 10 g dextrose, 1.0 g sodium thioglycollate, 6.0 g Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O, 2.5 g NaHSO<sub>3</sub>, 7.0 g lecithin, 5.0 g polysorbate 80 and 0.02 g bromocresol purple per L.

Prepare according to manufacturer's directions. Dispense 9 or 9.9 mL aliquot into tubes and sterilize by autoclaving at 121 ° C for 15 min. Final pH should be 7.6 ± 0.2 at 25 ° C. Use for aerobic plate count, L, dilutions.

### **H.4.3 Nutrient agar**

Contains 5.0 g pancreatic digest of gelatin 3.0 g beef extract, and 15.0 g agar per L. Prepare according to manufacturer's directions. Dispense into tubes and sterilize by autoclaving at 121 ° C for 15 min. Final pH should be 6.8 ± 0.2 at 25 ° C. Cool in inclined position to form a slant. Use for bacterial culture maintenance and inoculum preparation.

### **H.4.4 Y/M agar (yeast/malt extract)**

Contains 3.0 yeast extract, 3.0 g malt extract, 5.0 g peptone, 10.0 g dextrose, and 20.0 g agar per L. Prepare according to manufacturer's directions. Dispense into tubes and sterilize by autoclaving at 121 ° C for 15 min. Final pH should be 6.2 ± 0.2 at 25 ° C. Cool in slanted position. Use for yeast culture maintenance and inoculum preparation.

#### H.4.5 Potato Dextrose Agar (PDA)

Contains 200 g potato infusion, 20.0 g dextrose, and 15.0 g agar per L. Prepare according to manufacturer's directions. Dispense into tubes and sterile by autoclaving at 121 ° C for 15 min. Final pH should be  $5.6 \pm 0.2$  at 25 ° C. Cool in slanted position. Use for mould culture maintenance and inoculum preparation.

#### H.4.6 0.85 % saline

Dissolve 8.50 g NaCl in water and dilute to 1 L. Dispense into flasks or bottles and sterilize by autoclaving at 121 ° C for 15 min.

#### H.4.7 0.85 % saline with 0.05 % polysorbate 80

Dissolve 8.5 g NaCl in water, mix in 0.50 g polysorbate 80, and dilute to 1L. Dispense into suitable containers and sterilize by autoclaving at 121 ° C for 15 min.

#### H.4.8 Barium sulphate standard No 2

H.4.8.1 Prepare a 1.0 % BaCl<sub>2</sub> solution by dissolving 1.0 g BaCl<sub>2</sub>.2H<sub>2</sub>O in 100 mL water. Let this be referred to as solution 1.

H.4.8.2 Prepare a 1.0 % H<sub>2</sub>SO<sub>4</sub> solution by mixing 1.0 mL H<sub>2</sub>SO<sub>4</sub> in 100 mL water. Let this be referred to as solution 2.

H.4.8.3 Mix 0.2 mL of solution (1) with 9.8 mL solution (2) in screw-capped test tube. Cap tightly and store in the dark at room temperature.

#### H.4.9 Barium sulphate standard No. 7

Use solutions from H.4.8. Mix 0.7 mL of solution H.4.8.1, with 9.3 mL of solution H.4.8.1, in a screw capped test tube. Cap tightly and store in the dark at room temperature.

### H.5 Microorganisms

H.5.1 *Staphylococcus aureus* — ATCC 6538

H.5.2 *Staphylococcus epidermidis* — ATCC 12228

H.5.3 *Klebsiella pneumoniae* — ATCC 10031

H.5.4 *Escherichia coli* — ATCC 8739

H.5.5 *Enterobacter gergoviae* — ATCC 33028

H.5.6 *Pseudomonas aeruginosa* — ATCC 9027

H.5.7 *Burkholderia cepacia* — ATCC 25416

H.5.8 *Acinetobacter baumannii* — ATCC 19606

H.5.9 *Candida albicans* — ATCC 10231

H.5.10 *Aspergillus niger* — ATCC 16404

NOTE Environmental micro organisms (s) likely to be contaminants of concern during product manufacture or use be included as a separate inoculum. Predominant environmental microbes isolated during manufacturing, equipment cleaning, and sanitizing, or from related demonized water systems are used as supplemental test inocula).

## H.6 Product quality check

**H.6.1** Weigh 1.0 g product into a screw-capped culture tube containing 9.0 mL sterile neutralizing broth to make a 1:10 dilution. If necessary to disperse product, add ten to twenty 3 mm diameter glass beads to tube. Mix on vortex mixer until homogeneous.

**H.6.2** Pipette 1.0 mL of the 1:10 dilution into each of 4 sterile petri plates. Pour 15 to 20 mL sterile molten Lethen agar ( $45^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ) into each plate. Mix by rotating plates to disperse the dilution thoroughly. Let solidify.

**H.6.3** Invert and incubate 2 plates at  $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for 48 h and 2 plates at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for 5 days.

**H.6.4** Count the number of colonies on all plates, add, and multiply by 2.5 to determine the number of colony forming units per gram (cfu/g) in the product.

**H.6.5** Save plates to be used for the neutralization validation in M by refrigerating.

## H.7 Product preparation

**H.7.1** Measure 20-mL sterile saline into 4 sterile jars, H.3.1. Cap tightly and store at room temperature.

**H.7.2** Weigh 20 g product into each of 4 sterile jars, H.3.1. Cap tightly and store at room temperature.

## H.8 Bacterial inocula preparation

**H.8.1** Streak each bacteria culture, H.5.1 to H.5.10 onto a nutrient agar, H.3.3, slant. Incubate 48 at  $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . Wash each slant with 5.0 mL sterile saline, loosening the culture from the agar surface. Transfer the suspension into a sterile tube. Repeat the wash with second 5.0 mL aliquot of saline. Combine washes and mix on vortex mixer to disperse evenly.

**H.8.2** Adjust each wash with sterile saline to yield a suspension of ca 108 cfu/mL using Mc Farland BaSO<sub>4</sub> standard No. 2, H.4.8, direct microscopic count, turbidimetry, absorbance, or other method correlated to an aerobic plate count (APC), H.12. Perform an APC, H.12, on each suspension to confirm standardization.

## H.9 Fungal inoculum preparation

**H.9.1** Streak *C. albicans*, H.5.9, on 3 slants of Y/M agar, H.4.4. Incubate at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for 48 h. Wash each slant sequentially with 5.0 mL aliquot of sterile saline. Repeat with a second 5.0 mL aliquot of sterile saline. Combine washes to produce 10 mL suspension. Mix on Vortex mixer to disperse evenly.

**H.9.2** Adjust the wash with sterile saline to yield a suspension of ca 10<sup>7</sup> cfu/mL using a Mc Farland BaSO<sub>4</sub> standard No. 7, H.4.9, direct microscopic count, turbidimetry, absorbance, or other method that has been correlated to an APC, H.13. Perform an APC, H.13, on the suspension to confirm standardization.

**H.9.3** Streak *A.Niger*, H.5.10, on 5 slants of potato dextrose agar H.4.5. Incubate at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for 10 days. Dislodge mould spores by adding 5.0 mL sterile saline containing 0.05 % polysorbate 80 to each tube and vigorously rubbing the surface of the agar slant with a sterile swab. Repeat with a second 5.0 mL aliquot in each tube. Combine the 10 washes to produce 50 mL suspension. Filter into a sterile container through 3 to 5 layers of sterile gauze supported in funnel. Perform an APC, H.13, using appropriate dilutions. Adjust mould

suspension to ca 10<sup>7</sup> per mL using sterile saline. Use immediately or refrigerate at 2 ° C to 5 ° C for up to 1 month. Verify mould viability by an APC, H.13, before each use.

## H.10 Inoculum pools

**H.10.1** Pool equal parts of the *S. aureus* and *S. epidermidis* suspensions, H.7.2 in a sterile container to make inoculum pool 1: Gram-positive cocci.

**H.10.2** Pool equal parts of the *K. pneumoniae*, *E. coli* and *E. gergoviae* suspensions, H.7.2, in a sterile container to make inoculum pool 2: Gram-negative fermentors.

**H.10.3** Pool equal parts of the *P. aeruginosa*, *B. cepacia* and *A. baumannii* suspensions, H.7.2, in a sterile container to make inoculum pool 3: Gram-negative non-fermentors. H.5.10, suspensions in a sterile container to make inoculum Pool 4: Fungi.

**H.10.5** Use organism pools immediately or refrigerate them at 2 ° C - 5 ° C for more than 72 h.

## H.11 Inoculation

H.11.1 Inoculate each of the four 20.0 mL aliquots of sterile saline, H.6.1, with 0.2 mL of its respective inoculum pool, H.10.1 to H.10.4. Mix thoroughly. Use these suspensions to determine inoculum counts (see K).

**H.11.2** Inoculate each of the four 20 g product suspensions, F (b) with 2.0 mL of its respective inoculum pools H.10d). Mix thoroughly by shaking, Vortex mixing or stirring, so that each suspension contains 10<sup>6</sup> bacteria or 10<sup>5</sup> fungi per gram, evenly distribute throughout the product. Tightly close inoculated containers and store at ambient temperature (20 - 25 ° C).

## H.12 Sampling intervals

**H.12.1** Sample each inoculated saline suspension, H.10.1, for APC, H.12, within 1 h after inoculation to obtain inoculum count.

**H.12.2** Test each inoculated product, H.10.2 for APC, H.12, at 7, 14 and 28 days after inoculation to obtain product interval count.

## H.13 Aerobic Plate Count (APC)

**H.13.1** Mix suspension thoroughly, weigh 1.0 g product into screw-capped culture tube containing 9.0 mL sterile neutralizing broth for a 1:10 dilution. If necessary to disperse product, add 10 to 20 sterile 3 mm diameter glass beads to the tube. Mix on Vortex mixer until homogeneous.

**H.13.2** Aseptically pipette 0.1 mL of the 1: 10 dilution into 9.9 mL tube of neutralizing broth to obtain a 1: 1000 dilution. Vortex mix. Pipette 0.1 mL of the 1:1000 dilution into 9.9 mL neutralizing broth to obtain a 1: 100 000 dilution.

The number of dilutions may be decreased if previous counts microbial populations show reduction.

**H.13.3** Using a 2.2 mL pipette, aseptically pipette 1.0 and 0.1 mL aliquots from the 1: 10 dilution into duplicate petri dishes for the 1:10 and 1: 100 plates. If necessary, transfer duplicate 1.0 and 0.1 mL aliquots from the 1 000 dilution for plates 1:1 000 and 1:10 000, and from the 1: 100 000 dilution for plates 1: 100 000 and 1: 1000 000. Pour 15 to 20 mL sterile Lethen agar H.3.1, (45 ° C ± 2 ° C), into each plate. Mix by rotating the plates to disperse the suspension thoroughly, and let solidify.

**H.13.4** Invert bacterial plates and incubate at  $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . Examine bacterial plates after 48 h - 72h. Count in suitable range (30 - 300 colonies). If no countable plates fall in that range, count the plate(s) nearest that range showing distinct colonies. Average duplicate plates counts and express results as cfu/g of product.

**H.13.5** Invert and incubate fungal plates at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . Read fungal plates at 2 to 3 days and record results. Count plates in a suitable range (30 to 300 colonies). If no countable plates fall in that range, count the plate(s) nearest that range showing distinct colonies. Reincubate plates for another 2- 3 days. Read and record additional colonies. Add to previous results to obtain total counts. Average duplicate plate counts and record as cfu/g of products.

## **H.14 Neutralization check**

Make a 1:10 000 dilution in sterile saline of pools 1, 2 and 3, H.10.1 - H.10.3, and a 1: 1 000 dilution of pool 4, H.10.4. Streak each dilution for isolation with a  $10\ \mu\text{L}$  loop on the plates saved from H.6.5. If plates are not usable due to either desiccation or surface growth, repeat section H.6, and streak freshly prepared plates. Incubate as in H.13.4 to H.13.5.

## **H.15 Data analysis**

**H.15.1** Product quality check, H.6.4, must be found to contain,  $<100\text{ cfu/g}$  to proceed with the challenge test.

**H.15.2** Inoculum counts, H.11.1, should be between 1 to  $9.9 \times 10^6\text{ cfu/g}$  product for bacteria and 1 to  $9.9 \times 10^5\text{ cfu/g}$  product for fungi, or the test should be repeated with different dilutions.

**H.15.3** Neutralization check, H.13, must show significant growth of all pools to confirm adequate neutralization. A neutralizing broth other than D/E broth can be used. If neutralization does not occur, the test is invalid. Refer to references 4 to 6 for assistance.

**H.15.4** Calculate the percentage reduction:

$$\text{Reduction, \%} = \frac{\text{inoculum count} - \text{product interval count}}{\text{inoculum count}} \times 100$$

**H.15.5** The test product is considered adequately preserved if

a) Bacteria show at least 99.9 % (3 log) reduction within 1 week following challenge and remain at or below that level thereafter, and

b) Fungi show at least a 90 % (1 log) reduction within 1 week following challenge, and remain at or below that level thereafter. These criteria apply to freshly prepared formulations.

**Annex I**  
(normative)

**Total heavy metals**

The total amount of heavy metals shall be calculated by adding up the values obtained for lead (Annex D), arsenic (Annex E), and mercury (Annex F). The report shall be given in ppm.

PUBLIC REVIEW



## Bibliography

IS: 7670-1975 Specification for hair creams.

76/768/EEC The European Economic Community Cosmetics Directive.

).

PUBLIC REVIEW



PUBLIC REVIEW