RWANDA STANDARD

RS EAS 878: 2017

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Antibacterial bathing bar — Specification

Reference number
RS EAS 878: 2017

In order to match with technological development and to keep continuous progress in industries, Standards are subject to periodic review. Users shall ascertain that they are in possession of the latest edition

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National foreword

This Rwanda Standard is identical with the first edition 2017 of Regional Standard EAS 878/*2017 Antibacterial b*athing bar — *Specification.*

It was approved by the RSB Board of Directors in accordance with Standards Development Procedures, in line with the relevant provisions of ISO/IEC Guide 21-1 on Regional or National adoptions of International Standards and other International Deliverables.

ICS 71.100.40

EAS 878: 2017

ICS 77.100.40

EAST AFRICAN STANDARD

Antibacterial bathing bar — Specification

EAST AFRICAN COMMUNITY

First Edition 2017

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.

EAS 878 was prepared by Technical Committee EASC/TC 074, *Surface active agents*.

Introduction

Performance of soap has been primarily based on Total Fatty Matter (TFM).

Whereas the above is a fact, technological trends have shown that performance of soap can be enhanced by acceptable and safe surface active agents where TFM levels have been reduced.

In this standard the TFM levels have been reduced from that of antibacterial toilet soap with introduction of surface active agents whilst serving the same purpose. However this does not necessarily imply substitution of antibacterial toilet soap but rather an alternative and affordable soap product.

This standard therefore sets minimum requirements for performance and safety characteristics of antibacterial bathing bar.

Antibacterial bathing bar — Specification

1 Scope

This East African Standard specifies the requirements, sampling and test methods for solid antibacterial bathing bars.

This standard applies to antibacterial bathing bars supplied in the form of bars/cakes and produced from vegetable or animal oils or fats, fatty acids, or from a blend of all or part of these materials, with or without the addition of rosins or non-soapy surfactants.

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 377 (all parts), *Cosmetics and cosmetics products*

EAS 794, *Determination of the microbial inhibition of cosmetic soap bars and liquid hand and body washes — Test method*

EAS 814, *Determination of biodegradability of surfactants — Test method*

ISO 456, *Surface active agents — Analysis of soaps — Determination of free caustic alkali*

ISO 685, *Analysis of soap — Determination of alkali content and total fatty matter content*

ISO 4315, *Surface active agents -- Determination of alkalinity -- Titrimetric method*

3 Terms and definitions

For the purpose of this standard the following term and definition shall apply.

antibacterial bathing bar
product in the form of a bar or cake containing antibacterial agent and soap of fatty acids and/ or synthetic surface active agents listed in 4.1.1 as active ingredients and which is used for bathing purposes in soft and hard water

4 Requirements

4.1 Ingredients

4.1.1 Surfactants may be used in the manufacture of antibacterial bathing bars. If used, they shall conform to the relevant East African Standards. Recommended surfactants include:

a) Linear Alkyl Benzene sulphonates (LAS);

b) Alkyl Poly Glycosides (APG);

c) fatty alcohol sulphosuccinate; and

d) fatty alkanol amido sulphosuccinate.

4.1.2 Soaps of fatty acids, fatty acid ester sulphonates, fatty alkalonamide, fatty alcohol ethoxylates, sarcosinates, taurides, fatty isothionates, alpha olefin sulphonates, alcohol sulphates and amphoterics such as betaines and fatty alcohol ethoxy sulphate may be used in the manufacture of antibacterial bathing bar. When used, they shall conform to the relevant East African Standards.

4.1.3 In addition to the surfactants, the antibacterial bathing bar shall contain permitted antibacterial agent. The label shall clearly state the antibacterial agent used and its level. The antibacterial bathing bar may contain other ingredients such as electrolytes, structuring and processing aids, colouring matter, perfume, permitted antioxidants, preservatives, permissible germicides, super fatting agents, humectants, plant extracts and such additional substances as are declared on the label and shall conform to the requirements of all parts of EAS 377.

4.1.4 All ingredients shall be declared on the label following descending order in terms of quantity.

4.2 General requirements

4.2.1 The product shall:

a) be in the form of cakes or bars;

b) not be injurious to health when used in a manner and purpose meant for its use or under reasonably foreseen conditions;

c) not have an unpleasant odour;

d) be firm and smooth in texture; and

e) not contain any ingredients in amounts that are harmful to the human body and environment.

4.2.2 The colour of the cake or bar shall generally be uniform. For the case of genuinely mottled products, the colour may not be uniform.

4.2.3 All the ingredients used in the bathing bars shall comply with the requirements of all parts of EAS 377.

4.3 Specific quality requirements

Antibacterial bathing bars shall conform to the performance and safety requirements given in Table 1 when tested in accordance with the methods prescribed therein.

Table 1 — Performance and safety requirements

SL. No.	Characteristic	Requirement	Test method
(i)	Total fatty matter, % by mass, min.	50	ISO 685
(ii)	Lather, ml, min.	200	Annex A
(iii)	Mush (loss in mass due mushing on a wet surface), g/30 cm^2, max.	10	Annex B
(iv)	Freedom from grittiness	To pass the test	Annex C
(v)	Total alkalinity (as NaOH), % by mass, max.	1.0	ISO 685 ISO 4315
(vi)	Free caustic alkali (as NaOH), % by mass, max.	0.05	ISO 456
(vii)	Antibacterial activity test	To pass the test	EAS 794
(viii)	Triclosan (TCN) and Trichlorocarbanilide (TCC), % by mass, max.	1	Annex D
(ix)	Chloroaniline content, mg/kg, max.	10	Annex E
(x)	Rosins, as % of total fatty matter, max.	2	Annex F
(xi)	Biodegradability test	To pass the test	EAS 814
(xii)	Synthetic surface active agents, % by mass, max.	4	Annex G
NOTE Trichlorocarbanilide (TCC) is not heat stable and decomposes into chloroanilines on prolonged heating above 60 °C. If TCC is used in soap, the manufacturer should take care that such soap is not subjected to temperature above 60 °C during the entire manufacturing process or during storage.			

5 Packaging and labelling

5.1 Packaging

Antibacterial bathing bars shall be wrapped to protect them from damage and excessive loss or gain of moisture.

5.2 Labelling

Each antibacterial bathing bar pack shall be legibly and indelibly labelled either in English, Kiswahili or French or combination or any other language as agreed between the manufacturer and supplier with the following information:

a) name of the product as ‘Antibacterial bathing bar’;

b) manufacturer’s name and physical address;

NOTE The name, physical address of the distributor/supplier and trade mark may be added as required.

c) net content;

d) number of bars or cakes contained in the package;

e) all ingredients in descending order of quantity;

f) batch number or code number;

g) date of manufacture and best before date;

h) antibacterial agents used and their levels; and

i) country of origin.

6 Sampling

Sampling shall be done in accordance with Annex H.

Annex A
(normative)

Test for lather volume

A.1 General

Attention shall be paid to all details of the procedure in order to ensure consistent results. Care should be taken to invert the cylinder exactly as described.

A.2 Outline of the method

A suspension of the material in standard hard water is taken in a graduated cylinder and given 12 inversions under prescribed conditions. The volume of the foam formed is observed after keeping the cylinder for 5 min.

A.3 Reagents

A.3.1 Calcium chloride $CaCl_2.2H_2O$, AR

A.3.2 Magnesium sulphate $MgSO_4.7H_2O$, AR

A.3.3 Distilled water

A.4 Apparatus

A.4.1 Graduated cylinder — Glass stoppered with graduation from 0 ml - 250 ml, with 2-ml divisions. Overall height about 35 cm and the height of the graduated portion about 20 cm.

A.4.2 100-ml glass beaker

A.4.3 Thermometer of range 0 ºC – 110 ºC

A.5 Preparation of standard hard water

Dissolve 0.220 g of calcium chloride dehydrate and 0.246 g of magnesium sulphate heptahydrate in distilled water. Dilute to 5 l with distilled water.

NOTE This standard hard water has a hardness of approximately 50 mg/kg calculated as calcium carbonate.

A.6 Sample preparation

Cut away the outer edges of antibacterial bathing bar using a knife.

Using a stand up type of grater, grate up to 10 g – 15 g of the antibacterial bathing bar into small chips.

A.7 Procedure

A.7.1 Weigh 1 g of the grated chips accurately in a 100-ml glass beaker. Add 10 ml of the standard hard water. Cover the beaker with a watch glass and allow to stand for 30 min. The operation is carried out to disperse the antibacterial bathing bar.

A.7.2 Stir the contents of the beaker with a glass rod and transfer the slurry to a 250-ml graduated cylinder ensuring that not more than 2 ml foam is produced. Repeat the transfer of the residue left in the beaker with further portions of 20 ml of standard hard water ensuring that all the matter in the beaker is transferred to the cylinder.

A.7.3 Adjust the contents in the cylinder to 100 ml by adding sufficient standard hard water. Bring the contents of the cylinder to 30 ºC. Stir the contents of the cylinder with a glass rod or thermometer to ensure a uniform suspension.

A.7.4 As soon as the temperature of the contents of the cylinder reaches 30 ºC, stopper the cylinder and give it 12 complete inversions, each inversion comprising movements in a vertical plane, upside down and vice versa. After the 12 inversions, let the cylinder stand for 5 min. Take the following readings as shown in Figure A.1:

a) foam plus water (V_1 ml); and

b) water only (V_2 ml).

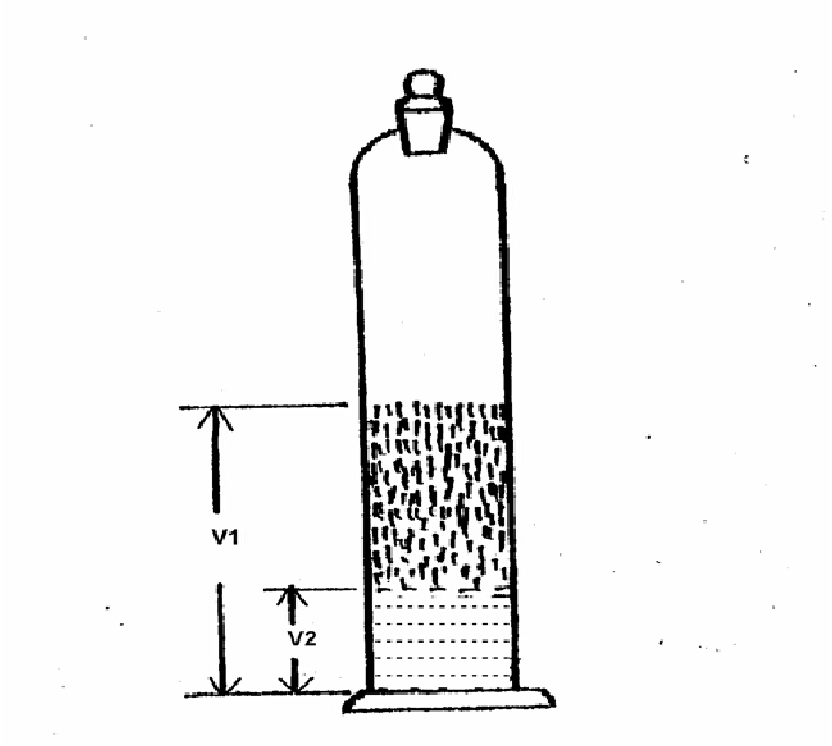

Figure A.1 — Measurement of foam

A.8 Calculation

Lather volume = V_1 - V_2

where

V_1 is the volume, in millilitres of foam and water; and

V_2 is the volume, in millilitres of water only.

Annex B
(normative)

Evaluation of the mushing properties

B.1 Principle

A test piece of defined size is cut from the sample bar to remove harder outer layers. The test piece is preconditioned by giving 18 x 180 degree twists under running water at 25 °C or in a bowl of water at 25 °C. The bar is left for six hours on a piece of fabric that has been wetted and drained of excess water. During the six hours the soap and cloth are covered to prevent drying. At the end of the test period mush is removed from the test piece face in contact with the cloth. Weight loss from the test piece is expressed as mush per 30 cm^2 of original surface area in contact with the cloth.

B.2 Equipment

B.2.1 For sample preparation

B.2.1.1 **Coarse kitchen cheese grater**

B.2.1.2 **Sharp thin blade knife or carpenters plane**

B.2.1.3 **Callipers or ruler** to ensure the sample dimensions

B.2.2 Other equipment/materials for the test

B.2.2.1 **Plastic or non-corrodible trays** which are suitable sized for the test piece. Plastic soap dishes 7 cm x 11 cm x 2 cm are quite suitable.

B.2.2.2 **Cotton cloth pieces** cut and folded to fit as a triple layer inside the trays. Normal, flat weave, cotton sheeting as used for bed sheets will be quite suitable.

B.3 Bar preparation

B.3.1 Three (3) individual bars of a type should be tested. A test piece is cut from each bar. The test piece should if possible have a working face (to be applied to the fabric) of (6 cm ±1 cm) x (4 cm ±1 cm).

B.3.2 All the bars in a set shall be cut to have the same face size. If the smallest of the range of bars to be tested at a given time is too small to allow a working face within these limits, then all bars should be cut to the maximum size possible from the smallest bar.

B.3.3 The longest axis of the test piece (6 cm ± 1 cm) should be from a direction parallel to the longest axis of the original bar sample.

B.3.4 The working face should be a fresh surface from the interior of the bar sample. The face opposite the working face should be identified by making a small hole with a sharp object. This enables the working face to be identified after the preconditioning step.

B.3.5 To cut the bar it is convenient to first trim it to the approximate size using a coarse kitchen cheese grater and then to make the final adjustments to a smooth surface with a sharp thin-bladed knife or carpenters plane. If a plane is used, it is better to move the bar over the plane blade.

B.4 Test procedure

B.4.1 The tray plus triple thickness of cloth is filled with demineralised water. The tray is then held vertically to drain the water from the cloth. The vertical position is maintained until water ceases to run from the dish in a continuous stream i.e. starts to drip.

B.4.2 The area of the working face of the test piece is measured (*A*).

B.4.3 The working face of the bar is placed onto the damp fabric and then the tray plus soap are covered for example, with a sealed plastic bag, to prevent water loss.

B.4.4 The covered test piece and holder are maintained at 25 °C for 6 h.

B.4.5 The mushed bar test piece is removed from the tray and is weighed (W_1).

B.4.6 Mush is removed from the working face of the bar test piece by scraping with the edge of a blunt sided spatula or plastic ruler.

B.4.7 The test piece is reweighed (W_2) and the amount of mush removed is calculated as in B.5. The mush is expressed as grams per 30 cm^2 of original test piece surface area.

NOTE The procedure for weighing the bar and removing the mush will take some minutes. During that time the remaining bars will continue to form mush. While this time is not critical for a set of three test pieces from a given product, if more than one product is under test it is advised to stagger the start of the test for the second product. This will give adequate time to complete work on the first set before the 6-hour storage time of the subsequent set is completed.

B.5 Calculation

The mush of bathing bar shall be expressed as follows:

Weight of mush, W (grams) = $W_1 - W_2$

Surface area of bar, A (square centimetres) = (width x breadth)

$$\text{Mush} = \frac{W \times 30}{A} \text{ grams per } 30 \text{ cm}^2$$

B.6 Criteria for conformity

B.6.1 The test is done with three (3) separate samples of each product type, and the mean value from three samples is quoted (X). The range of values (R) is quoted as the difference between the highest and lowest values obtained for a given product type.

B.6.2 The sample lot of products shall be declared as conforming to the requirements for this standard if $X + 0.6 \times R$ is less than the maximum value given in Table 1.

Annex C
(normative)

Determination of grittiness

C.1 Procedure

C.1.1 Hold the antibacterial bathing bar under a smooth stream of running water at a temperature of 30 °C and gently rub the two sides of the bar on the palm of one hand for one minute each side.

C.1.2 Immerse the soap in a bowl containing 5 l of water at 30 °C and gently rub two opposite bar faces with the palm of one hand for 30 s (15 s per bar face). Remove the bar from the water and continue to gently rub the two opposite bar faces for a further 30 s (15 s per face).

C.1.3 Allow the used bar to dry in the open for 4 h and examine the surface. A set of three samples will be tested for each product.

NOTE 1 Hands will become hydrated and insensitive with prolonged immersion in water. Testers should wait 15 min between testing every three sets of products (nine grit tests).

NOTE 2 If using a bowl rather than running water use fresh water after testing every set of three samples.

C.2 Criteria for conformity

The performance criteria are:

a) during manipulation under running water, the bathing bar shall not have a visibly rough surface and shall feel smooth to the touch; and

b) no gritty particles shall be observed on the surface of the dried bar 4 h after the washing test.

Annex D
(normative)

Determination of TCC and TCN by HPLC

D.1 Principle

TCC and TCN are antibacterial agents, which are separated from other components in soap by normal phase or reverse phase liquid chromatography, detected spectrophotometrically and quantified by comparison with standard TCC and TCN. The method can estimate as low as 1 mg/kg of the above compounds.

Procedures for both normal and reverse HPLC have been described and provide the option to use either method whichever is available to the users. Both methods are comparable.

D.2 Normal phase HPLC

D.2.1 Reagents

D.2.1.1 **Iso-octane,** HPLC grade

D.2.1.2 **Iso-propanol (2 propanol),** HPLC grade

D.2.1.3 **Hexane,** HPLC grade

D.2.1.4 **Standard TCC,** 99 % pure

D.2.1.5 **Standard TCN,** 99 % pure

D.2.2 Apparatus

D.2.2.1 **High performance liquid chromatography**, consisting of a pump, a sample injector of fixed volume with UV detector having variable wavelengths and a recorder

D.2.2.2 **Standard volumetric flasks**

D.2.2.3 **Pipettes**

D.2.2.4 **Magnetic stirrer**

D.2.2.5 **Millipore filter apparatus** with 0.5-µ filter

D.2.2.6 **Column**

D.2.2.6.1 **Silica column,** stainless steel, 25 cm x 0.46 cm packed with Normal Phase – Silica 5 µ (Lichrosorb Si-60)

D.2.2.6.2 **Cyano column,** stainless steel 25 cm x 0.40 cm packed with (Lichrospher 100) cyano 5 µ

NOTE Either of the above columns can be used depending on the availability.

D.2.2.7 **Mobile Phase**

D.2.2.7.1 For silica column, transfer 20 ml of iso-propanol into a 500-ml volumetric flask and make up to mark with iso-octane and mix well. Assemble millipore filter apparatus and filter the solvent system prior to use.

D.2.2.7.2 For cyano column, transfer 50 ml of HPLC grade iso-propanol (2-propanol) into a 500-ml volumetric flask, fill up to the mark with hexane and mix well. Assemble millipore filter apparatus and filter the solvent system prior to use.

D.2.2.8 HPLC conditions

a) Detector wavelength: 280 nm

b) Flow rate: 0.5 ml/min

c) Injection volume: 20 µl

d) Retention time

e) Silica column

- TCN -7.5 min
- TCC -19.2 min

f) Cyano column

- TCN -4.0 min
- TCC -7.5 min

D.2.3 Procedure

D.2.3.1 Standard preparation (see note under D.3.4)

Weigh accurately 25 mg of triclosan (TCN) and 25 mg of TCC into a 100-ml volumetric flask and make up to volume with the mobile phase and mix well. Pipette 1.0 ml of this solution in a 50-ml volumetric flask and dilute with mobile phase. Final concentration of TCC and TCN is 250 µg/50 ml (5.0 mg/kg).

D.2.3.2 Sample preparation

Weigh accurately 1 g of homogenized sample into a 100-ml standard flask, and dilute to the mark with mobile phase. Pipette 10 ml of the supernatant liquid to a 50-ml volumetric flask, dilute with mobile phase, to the mark, and filter through 0.45-µm filter.

D.2.3.3 Chromatography

Equilibrate the column, maintained at a temperature of 30 °C, with the mobile phase with a flow rate of 0.5 ml/min for *iso*-octane-iso-propanol mobile phase and 1.0 ml/min for hexane–*iso*-propanol mobile phase for 30 min. Set the wavelength at 280 nm. Inject 20 µl of standard solution and then sample solutions. Measure the area of the peaks of respective retention time for standard and sample.

D.2.4 Calculation

The TCN and TCC contents shall be expressed as follows:

$$\text{TCN, percent by mass} = \frac{\text{Area of sample for TCN} \times \text{Concentration of standard TCN}}{\text{Area of standard TCN} \times \text{Concentration of sample}} \times 100$$

$$\text{TCC, percent by mass} = \frac{\text{Area of sample for TCC} \times \text{Concentration of standard TCC}}{\text{Area of standard TCC} \times \text{Concentration of sample}} \times 100$$

D.3 Reverse phase

D.3.1 Reagents

D.3.1.1 **Methanol,** HPLC grade

D.3.1.2 **Sodium Dihydrogen Phosphate Monohydrate,** Chemical grade

D.3.1.3 **Standard TCC**

D.3.1.4 **Standard TCN (TCS)**

D.3.1.5 **10 % phosphate solution**

D.3.2 Apparatus

D.3.2.1 **Column – Octyldimethylsilyl (C-DB) Supercosil LC-8-DB** – 15 cm x 4.6 mm. 5 µ.

D.3.2.2 **Mobile Phase** – MeOH/0.01 M Phosphate buffer 62:38 v/v.

To prepare 0.01 M Phosphate buffer solution, dissolve 1.38 g sodium dihydrogen phosphate monohydrate in 1 000 ml of distilled water. Adjust the pH of the mobile phase to 3.0 using 10 % phosphate solution.

D.3.3 Procedure

D.3.3.1 Standard preparation (see Note under D.3.4)

D.3.3.1.1 Weigh accurately about 90 mg TCN. Dissolve in methanol and make up to 1 000-ml volumetric flask with methanol.

D.3.3.1.2 Weigh about 110 mg of TCC, dissolve well with methanol, and make up the volume to 1 000 ml.

D.3.3.1.3 Accurately pipette 10 ml of the solution prepared in (see D.3.3.1.1) into the (see D.3.3.1.2) volumetric flask containing TCC. And make up to the volume with methanol. Then accurately pipette 5-ml of the solution into a 50-ml volumetric flask. Make up to the volume with methanol. Filter this standard solution through 0.45-µm filter.

D.3.3.2 Sample preparation

Weigh accurately about 1.0 g of product, dissolve in methanol and make up to 100 ml in a volumetric flask with methanol. Filter this sample solution through 0.45-µm filter.

D.3.3.3 HPLC conditions

a) **Detector wavelength**: 280 nm

b) **Column temperature**: 35°C

c) **Flow rate**: 1.0 ml/min

d) **Injection volume**: 10 µl

Prepare the standard solution and the sample solution at the same time. Inject the standard solution three times and calculate the average of each ingredients peak count. Inject 10 µl the sample solution and determine each ingredients percentage by the calculation shown below.

D.3.4 Calculations

The TCN and TCC contents shall be expressed as follows:

$$\text{TCN percent by mass} = \frac{M_s \times A_r \times F}{A_s \times M_t \times 100}$$

$$\text{TCC percent by mass} = \frac{M_s \times A_r \times F}{A_s \times M_t \times 100}$$

where

M_s is the mass, in grams, of the standard;

A_s is the averaged peak of the area of the standard;

M_t is the mass, in grams, of the test sample;

A_r is the peak area of the test sample; and

F is the purity, as a percentage of the standard.

NOTE Both TCC and TCN are photosensitive, hence standards should be freshly prepared.

Annex E
(normative)

Determination of chloroaniline

E.1 Principle

The chloroanilines are extracted from soap with dimethyl sulfoxide and diazotized with nitrous acid. The reaction products are then coupled with N-1-(naphthyl) ethylenediamine hydrochloride to produce coloured compounds which are estimated spectrophotometrically.

E.2 Safety precautions

Dimethyl sulfoxide (DMSO) is readily absorbed into the skin. Inhalation or skin penetration shall be avoided. DMSO should never be pipette using mouth. Always use pipette bulb. The standard chloroanilines and N-1-(naphthyl)–ethylenediamine hydrochloride shall not be allowed to come into contact with the skin. If they should, then wash the contaminated parts thoroughly with soap and water.

A supply of diluted sodium hypochlorite should be at hand at all times to deal with accidental spillages of chloraniline solution. Spillage on laboratory surface should be treated immediately with the sodium hypochlorite solution, followed by water.

E.3 Reagents

E.3.1 **Dimethyl sulphoxide (DMSO)**, AR grade

E.3.2 **Hydrochloric acid**, concentrated (specific gravity – 1.18)

E.3.3 **Sodium nitrite**, 0.4 %w/v analytical grade, freshly prepared (aqueous)

E.3.4 **Ammonium sulphamate**, 2 % w/v solution freshly prepared, (aqueous)

E.3.5 **N-1-(naphthyl) Ethylene**, 0.1 % w/v solution diamine hydrochloride freshly prepared (aqueous)

E.3.6 **n-Butanol**, AR grade

E.3.7 **Sand**, acid purified 40-µ – 100-µ mesh

E.3.8 **Solvent mixture**

a) DMSO 5 volumes

b) *n*-butanol 2 volumes

c) distilled water 2 volumes

d) hydrochloric acid 1 volume

Mix *n*-butanol, water and HCl. Cool the mixture and add DMSO (5 volumes).

E.3.9 **4-chloroaniline and 3, 4-dichloroaniline**, AR grade

E.4 Apparatus

E.4.1 Spectrophotometer, suitable for use at 554 nm

E.4.2 Cuvettes, glass (matched pair) 10 mm

E.4.3 Water bath, thermostatically controlled at 25 °C

E.4.4 Stopwatch

E.4.5 Standard laboratory glassware

E.4.6 Filter paper, Whatman No. 541

E.5 Procedure

E.5.1 Preparation of calibration curve

E.5.1.1 Dissolve 0.349 8 g of 3, 4-dichcoroaniline and 0.275 3 g of 4-chroroaniline in solvent mixture (see E.2.8) in a 250-ml amber volumetric flask. Dilute to mark with solvent mixture. 1 ml = 2.5 mg mixed chloroanilines (stock solution).

E.5.1.2 Dilute this stock solution with solvent mixture as given below:

a) Take 5 ml of stock solution and dilute it to 250 ml with mixture.

   (1 ml = 50 µg mixed chloroanilines); and

b) Take 5 ml of the above solution (see E.5.1.2 (a)) and further dilute to 250 ml with solvent mixture.

   (1 ml = 1 µg mixed chloroanilines).

Use this solution for preparation of calibration curve.

Transfer using a burette 0 ml, 1 ml, 2 ml, 5 ml, 10 ml, 20 ml, 40 ml into 50-ml amber volumetric flasks.

E.5.1.3 From a burette, add sufficient solvent mixture to make total volume to 40-ml in each flask. The flasks are incubated in a water bath at 25 ºC for 20 min. After exactly 20 min, add 2 ml of reagent (see E.3.3) into each flask and return them to the water bath for exactly 10 min (measure with a stop watch). Then add 2 ml of reagent (see E.3.4) into each flask and return them to the water bath for exactly 10 min. Swirl the flask occasionally. Then add 2 ml of reagent (see E.3.5) into each flask and remove them from the water bath. Dilute to volume with distilled water, mix and allow to stand for 30 min. Measure absorbance at 554 nm against the blank solution as prepared in E.5.1.4.

E.5.1.4 For the preparation of blank solution, take 40 ml of solvent mixture in a 50-ml amber volumetric flask. Incubate the flask in water bath at 25 ºC for 20 min. After exactly 20 min, add 2 ml of reagent (see E.3.3) into the flask and return it to the water bath for exactly 10 min. Then add 2 ml of reagent (see E.3.4) into the flask and return it to the water bath for exactly 10 min (swirl the flask occasionally).

Then add 2 ml of reagent (see E.3.5) into the flask and remove it from the water bath. Dilute to volume with distilled water, mix and allow to stand for 30 min. Use this blank solution for preparation of calibration curve only.

E.5.1.5 Prepare a graph by plotting weight (µg) of chloroanilines contained in each 50-ml flask against absorbance. The linear calibration will pass through the origin/or determine the average absorbance (AA) of 1 µg of mixed chloroanilines by dividing sum of absorbances of all different aliquots of the standard by sum of micrograms of chloroanilines in all different aliquots of standard.

E.6 Determination of chloroanilines

E.6.1 Weigh to the nearest milligram, 3.0 g - 3.5 g of finely grated antibacterial bathing bar and add 10.0 g – 15.0 g of acid purified sand. Transfer quantitatively the sample and the sand into a mortar and grind the mixture thoroughly with a pestle to give a homogenous mass.

Transfer the mass to a previously weighed 250-ml flat bottom flask quantitatively and reweigh. Add DMSO (100 ml), stopper firmly and attach the flask to an automatic shaker. Shake for 1 h.

Filter the DMSO extract through Whatman No.541 into a 250-ml amber volumetric flask. Wash the flask and filter paper with small aliquots of DMSO. Allow the filtrate to drain completely, dilute to volume with DMSO and mix. Transfer 20-ml DMSO extract into 50-ml amber volumetric flask.

Add 20 ml of solvent mixture. The flask is incubated in a water bath at 25 ºC for 20 min. After exactly 20 min, add 2 ml of reagent (see E.3.3) into the flask and return it to the water bath for exactly 10 min (measure with a stopwatch). Then add 2 ml of reagent (see E.3.4) into the flask and return it to the water for exactly 10 min (swirl the flask occasionally). Then add 2 ml of reagent (see E.3.5) into the flask and remove it from the water bath. Dilute to volume with distilled water, mix and allow to stand for 30 min. Read the absorbance at 554 nm against blank (prepared as below).

E.6.2 Prepare the blank solution by mixing 20 ml of DMSO extract of sample and 20 ml of solvent mixture in a 50-ml amber volumetric flask. Incubate the flask in a water bath at 25 ºC for 20 min.

After exactly 20 min, add 2 ml of distilled water into the flask and return it to the water bath for exactly 10 min. Then add 2 ml of reagent (see E.3.4) into the flask and return it to the water bath for exactly 10 min (swirl the flask occasionally).Then add 2 ml of reagent (see E.3.5) into the flask and remove it from the water bath. Dilute to volume with distilled water, mix and allow to stand for 30 min. Use the solution as a blank for reading sample only.

E.6.3 Deduce the amount of chloroaniline (µg) from the calibration graph curve. The determination shall be completed in one day.

E.7 Calculations

E.7.1 Determine the amount of mixed chloroanilines in the aliquot of test solution from the calibration graph.

$$\text{Chloroaniline content (in mg/kg)} = \frac{250(M + M_1)M_3}{20M_2M}$$

where

M is the mass, in grams, of bar,

M_1 is the mass, in grams, of sand,

M_2 is the mass, in grams, of bar and sand transferred to the flask,

M_3 is the mass, in micrograms, of mixed chloroanilines found from calibration graph/or it can be calculated as given below:

$$M_3 = \frac{\text{Mass of the sample}}{\text{Average absorbance of 1 µg mixed chloroanilines (AA)}}$$

Where

$$AA = \frac{\text{Sum of the OD of the standards}}{\text{Sum of concentration of standard chloroanilies in micrograms}}$$

$$\text{Weight of soap actually used, in grams} = \frac{M_2 M}{M + M_1}$$

Annex F
(normative)

Determination of rosins

F.1 General

F.1.1 Colophonium (commercial rosins) only shall be considered as rosin for the purpose of this standard. The mean equivalent weight of the rosin acid is taken as 346.

F.1.2 The method described in this test gives results approximately one percent higher than the actual amount of rosin present. As a result, the percentage of actual rosin acids present is one less than the percentage of rosin acids found experimentally and hence minus one in the formula.

F.2 Reagents

F.2.1 Dilute Sulphuric Acid — 30 % (w/v) obtained by cautiously adding 16 ml of sulphuric acid, specific gravity 1.84 to 70 ml of water.

F.2.2. Beta-naphthalene Sulphuric Acid Solution — $C_{10}H_7SO_3H$) — Obtained by dissolving 40 g of the chemical in one litre of chemically pure, absolute methyl alcohol.

F.2.3 Standard Alcoholic Potassium Hydroxide Solution — Approximately 0.2 N in 95 % (v/v) ethyl alcohol or in rectified spirit, accurately standardized. Since alcohol is volatile, frequent restandardization is necessary.

F.2.4 Phenolphthalein Indicator — Obtained by dissolving 1 g in 100 ml of 95 % (v/v) ethyl alcohol.

F.3 Procedure

F.3.1 Dissolve 10 g - 50 g of the sample in about 500 ml of hot water. Add 10 ml - 50 ml of the dilute sulphuric acid to split the bar, keep in steam-bath until the fatty matter separates as a clear layer and siphon off the lower aqueous acid layer. Add 300 ml of hot water, boil gently for a few minutes and siphon off the aqueous layer. Repeat the washing with hot water several times until the wash liquor is free of mineral acids. Complete the acidification and washing in as a short period as possible, keeping the beaker covered to prevent oxidation of the acids. Remove the last traces of water from the fatty acids through one or two thickness of filter paper and dry at 105 ˚C ± 2 ˚C for 45 min - 50 min.

F.3.2 Weigh accurately 2 g of the mixture of fatty and rosin acids into an esterification flask and add 25 ml of beta-naphthalene sulphonic acid solution. Boil gently under a reflux condenser for 30 min, adding a few glass beads to ensure smooth boiling. Cool the contents of the flask and titrate immediately with standard alcoholic potassium hydroxide solution, using 0.5 ml of phenolphthalein indicator. The end point is reached when the pink colour persists for 30 s.

F.3.3 Conduct simultaneously a blank determination with 25 ml of the etherifying agent alone.

F.4 Calculation

F.4.1 Rosin acids in fatty matter shall be expressed as follows:

a) Rosin in fatty acids, percent by mass, uncorrected = $\frac{34.6(S-B)N}{M}$

where

S is the volume in ml of standard alcoholic potassium hydroxide solution required for the material,

B is the volume in ml of standard alcoholic potassium hydroxide solution required for the blank,

N is the normality of alcoholic potassium hydroxide, and

M is the mass in g of the material taken for the test.

The method described above gives results approximately one percent higher than the actual amount of rosin present. As a result, the actual percentage of rosin acids present is one less than the percentage of rosin acids found experimentally.

b) Rosin in fatty acids, percent by mass, corrected = Rosin in fatty acids, percent by mass, uncorrected – 1.0.

NOTE 1 — The mean equivalent mass of the rosin acids is taken as 346.

NOTE 2 — When the quantity of rosin, expressed as percent by mass, is less than 5 in the bars, the results by this method are not so accurate as with bars containing higher rosin content. This method is also liable to give erroneous results with certain types of carbolic soaps containing high boiling tar acids and with other germicidal soaps, for example, soaps containing hexachlorophene.

F.4.2 In all cases where the rosin content is found to be less than 5 %, the actual presence or absence of rosin should be checked qualitatively by the Liebermann-Storch test,

F.4.2.1 Reagents

a) **Acetic anhydride** — pure.

b) **Dilute sulphuric acid** — relative density 1.53.

F.4.2.2 Procedure

Transfer 1 ml - 2 ml of the sample of fatty acids to a test-tube, treat with 5 ml - 10 ml of acetic anhydride and warm on a steam-bath. After cooling, pour 1 ml - 2 ml into a white porcelain dish and allow a drop or two of sulphuric acid to run down the side of the vessel. If rosin is present, a fugitive violet colouration changing to a brownish tinge is immediately produced at the margin of contact of the reagents. Check the test with a sample of fatty acids to which a small amount of rosin has been added.

Annex G
(normative)

Determination of active detergent content

G.1 Outline of the method

When equivalent amounts of cationic and anionic detergents are present in a two-phase mixture of water and chloroform, methylene blue will colour the two phases to the same degree. Sodium alkyl benzene sulphonate and sodium lauryl sulphate or any other detergent can be titrated with a standard solution of cetyl trimethyl ammonium bromide.

G.2 Reagents

Weigh 1.5 g ± 0.001 g of cetyl trimethyl ammonium bromide into a 250-ml beaker. Add 100 ml of distilled water and stir until dissolved. Transfer quantitatively to a one-litre volumetric flask and make to volume. Mix thoroughly and standardize against solution B (see G.2.1).

G.2.1 Anionic solution (Solution B)

Weigh accurately such amount of standard alkyl sulphate of known combined SO_3 or active content so as to give exactly 0.320 g of combined SO_3 into a 250-ml beaker. Dissolve in 100 ml - 200 ml of warm water. Transfer quantitatively to one-litre volumetric flask and make to volume with water at room temperature. Mix thoroughly. This is the primary standard against which solution A, is standardized. Solution B is 0.004 N.

G.2.2 Methylene blue indicator

Dissolve 0.1 g of methylene blue in 100 ml of water. Transfer 30 ml of this solution to a one-litre flask. Add 500 ml of water, 6.8 ml of concentrated sulphuric acid, 50 g of ($NaH_2PO_4 \cdot H_2O$) sodium dihydrogen phosphate monohydrate and shake until dissolution is complete. Dilute to the mark.

G.2.3 Chloroform

Analytical reagent grade.

G.3 Procedure

G.3.1 Weigh accurately a sample of sufficient size to give approximately 0.320 g of combined SO_3 into a 250-ml beaker. Sample size is crucial (see Note). Use 700 ml - 800 ml of warm water to transfer quantitatively to a one-litre volumetric flask. Warm on steam bath and shake gently until the sample is dissolved and solution is clear. Cool, dilute to the mark and mix thoroughly.

NOTE The titration value V should be as near as to 10 ml as possible, say between 8 ml and 12 ml but never outside 5 ml and 15 ml.

G.3.2 Pipette 10.0 ml of the sample solution into a 100-ml glass stoppered cylinder (25 mm x 300 mm). Add 25.0 ml ± 0.5 ml of methylene blue solution and 10 ml ± 0.5 ml of chloroform (see Note). Titrate with solution A to the correct end point, shaking the cylinder carefully after such addition to avoid emulsion and maintaining temperature within prescribed limits of 20 °C - 30 °C by immersion in water bath, if necessary. As the end point is approached, the rate of transfer of colour increases and solution A shall be added dropwise with vigorous shaking after each addition. If the approximate titration is known, 80 % of the required titrating

solution should be added before shaking since this avoids emulsion formation. Application of vacuum to the titration cylinder may help to break some emulsions, if formed. The end point is reached when both layers have same colour intensity. The end point is very sharp and 0.05 ml will cause a distinct change in colour distribution at or near the equivalence point.

NOTE The titration value *V* should be as near to 10 ml as possible, say between 8 ml and 12 ml but never outside 5 ml and 15 ml.

G.3.3 Pipette 10.0 ml of the sample solution into a 100-ml glass stoppered cylinder (25 mm x 300 mm). Add 25.0 ml ± 0.5 ml of chloroform (see Note). Titrate with solution A to the correct end point, shaking the cylinder carefully after such addition to avoid emulsion and maintaining temperature within prescribed limits of 20 ºC - 30 ºC by immersion in water bath if necessary. As the end point is approached, the rate of transfer of colour increases and solution A shall be added dropwise with vigorous shaking after each addition. If the approximate titration is known, 80 % of the required titrating solution should be added before shaking since this avoids emulsion formation.

Application of vacuum to the titration cylinder may help to break some emulsions, if formed. The end point is reached when both layers have same colour intensity. The end point is very sharp and 0.05 ml will cause a distinct change in colour distribution at or near equivalence point.

NOTE The volume of methylene blue solution and chloroform may be changed if found advantageous provided the same volumes are used in standardizing solutions A and B.

G.3.4 Calculation

G.3.4.1 The percent combined SO_3 shall be expressed as follows:

$$\% \text{ combined } SO_3 = \frac{V \times N \times 8.0}{M}$$

where,

V is the volume in millilitres, of solution A used in the titration;

N is the normality of solution A; and

M is the mass in grams, of the sample in the aliquot.

G.3.4.2 The percent active detergent content shall be expressed as follows:

Percent active detergent content = percent combined SO_3 x Molecular weight of active detergent.

NOTE The molecular weight of active detergent should be supplied by the manufacturer on request.

G.4 Alternative method for determination of active detergent content

G.4.1 Field of application

This method is to be used only if the first method in G.1 fails to work on the product.

This method is applicable to the analysis of alkylbenzene sulphonates, alkyl sulphonate, sulphates and hydroxy-sulphates, alkylphenol and fatty alcohol ethoxysulphates and dialkyl sulphosuccinates and to the determination of active materials containing one hydrophilic group per molecule.

G.4.2 Principle

Determination of anionic-active matter in a medium consisting of an aqueous and chloroform phase by volumetric titration with a standard cationic-active solution (benzethonium chloride), in the presence of an indicator which consists of a mixture of a cationic dye (dimidium bromide) and an anionic dye (acid blue 1).

G.4.3 Reagents

G.4.3.1 Distilled water

G.4.3.2 Chloroform, (specific gravity = 1.48, distilling between 59.5 ºC and 61.5 ºC)

G.4.3.3 Sulphuric acid, 2.5 M solution

G.4.3.4 Sulphuric acid, 0.5 M solution

G.4.3.5 Sodium hydroxide, 1.0 M standard volumetric solution

G.4.3.6 Sodium lauryl sulphate (sodium dodecyl sulphate) ($CH_3(CH_2)_{11}OSO_3Na$), 0.004 M standard volumetric solution. Check the purity of the sodium lauryl sulphate and simultaneously prepare the standard solution.

G.4.3.6.1 Determination of purity of sodium lauryl sulphate — Weigh, 5 g ± 0.2 g of the product into a 250-ml round bottom flask with ground glass neck. Add exactly 25 ml of the sulphuric acid solution (G.4.3.4) and reflux into a water condenser.

During the first 5 min - 10 min, the solution will thicken and tend to foam strongly; control this by removing the source of heat and swirling the contents of the flask.

In order to avoid excessive foaming, instead of refluxing the solution may be left on a boiling water bath for 60 min.

After a further 10 min the solution clarifies and foaming ceases. Reflux for further 90 min. Remove the source of heat, cool the flask and carefully rinse the condenser with 30 ml of ethanol followed by water.

Add a few drops of the phenolphthalein solution (G.4.3.8) and titrate the solution with the sodium hydroxide solution (G.4.3.5).

Carry out a blank test by titrating 25 ml of the sulphuric acid solution (G.4.3.4) with the sodium hydroxide solution (G.4.3.5).

The purity of the sodium lauryl sulphate, expressed as a percentage,

$$= \frac{28.84\,(V_1 - V_O)\;M_O}{M_1}$$

where,

V_0 is the volume, in millilitres, of sodium hydroxide solution used for the blank test;

V_1 is the volume, in millilitres, of sodium hydroxide solution used for the sample;

M_1 is the mass, in grams, of the sodium lauryl sulphate to be checked; and

M_0 is the exact molarity of the sodium hydroxide solution.

G.4.3.6.2 Weigh 0.004 M sodium lauryl sulphate standard volumetric solutions. Weigh, to the nearest 1 mg between 1.14 g and 1.16 g of sodium lauryl sulphate and dissolve in 200 ml of water. Transfer to a ground glass stoppered 1-l one-mark volumetric flask and dilute to the mark with water.

Calculate the molarity, M_1, of the solution by means of the solution by means of the formula:

$$M_1 = \frac{m_2 \text{ x } purity\ (\%)}{288.4 \text{ x } 100}$$

where,

m_2 is the mass in grams of sodium lauryl sulphate.

G.4.3.7 Benzethonium chloride 0.004 M standard volumetric solution

Weigh, to the nearest 1 mg, between 1.75 g and 1.85 g benzethonium chloride and dissolve in water. Transfer to a ground glass-stoppered 1-l one-mark volumetric flask and dilute to the mark with water.

NOTE In order to prepare a 0.004 M solution, dry the benzethonium chloride at 105 °C, weigh 1.792 g, to the nearest 1 mg, dissolve in water and dilute to 1 l.

G.4.3.8 Phenolphthalein, ethanolic solution containing 10 g/l. Dissolve 1 g of phenolphthalein in 100 ml of 95 % (v/v) ethanol.

G.4.3.9 Mixed indicator

G.4.3.9.1 Stock solution

Weigh 0.5 g ± 0.005 g dimidium bromide into a 50-ml beaker, and 0.025 g ± 0.005 g of acid blue 1 into a second 50-ml beaker.

Add between 20 ml and 30 ml of hot 10 % (v/v) ethanol to each beaker. Stir until dissolved and transfer the solutions to a 250-ml one mark volumetric flask. Rinse the beakers into the volumetric flask with ethanol and dilute to the mark with 10 % (v/v) ethanol.

G.4.3.9.2 Mixed acid indicator solution

Take 20 ml of the stock solution prepared above, put it in a 500-ml one-mark volumetric flask. Add 200 ml of water and 20 ml of 2.5 M sulphuric acid (D.4.3.3) mix and dilute to the mark with water. Store away from direct sunlight.

G.4.4 Apparatus

Ordinary laboratory apparatus, and

a) bottles, 200-ml, glass stoppered, or measuring cylinders, glass stoppered.

b) burettes, 25-ml and 50-ml.

c) one-mark volumetric flask, 1-l capacity glass stoppered.

d) one-mark pipette, 25-ml.

G.4.5 Procedure

G.4.5.1 Standardization of benzethonium chloride solution

By means of the pipette transfer 25 ml of the 0.004 M sodium lauryl sulphate solution to a bottle or measuring cylinder, add 10 ml of water, 15 ml of the chloroform and 10 ml of the mixed indicator solution.

Titrate with the 0.004 M benzethonium chloride solution. Stopper the bottle or measuring cylinder after each addition and shake well. The lower layer will be coloured pink. Continue the titration with repeated vigorous shaking. As the end point approaches, the emulsions formed during shaking tend to break easily continue the titration drop by drop, shaking after each addition of titrant, until the end point is reached. This is at the moment when the pink colour is completely discharged from the chloroform layer, which becomes a faint greyish blue.

The molarity, M, of the benzethonium chloride solution is given by the formula:

$$M = \frac{M_1 \text{ x } 25}{V_2}$$

where,

M_1 is the molarity of the sodium lauryl sulphate solution; and

V_2 is the volume, in millilitres of benzethonium chloride added.

G.4.5.2 Determination

Weigh to the nearest 1 mg a sample of 30 g; dissolve the test portion in water. Add a few drops of the phenolphthalein solution and neutralize to a faint pink colour with the sodium hydroxide solution or sulphuric acid solution as required.

Transfer to one-litre volumetric flask and dilute to the mark with water. Mix thoroughly and, by means of the pipette transfer 25 ml of this solution to a bottle or measuring cylinder, add 10 ml of water, and 15 ml of chloroform. Titrate with the benzethonium chloride solution as described in G.4.5.1.

G.4.6 Expression of results

The content as a percentage by mass, of anionic-active matter

$$\frac{V_3 \times M \times 1000 \times M_0 \times 100}{25 \times 1000 \times M_0} = 4V_3M$$

The amount of active matter, expressed in milliequivalents per gram,

$$\frac{40 \times V_3 \times M_1}{M_0}$$

where,

M_0 is the mass, in grams, of the test portion;

M is the relative molar mass of anionic-active matter;

M_1 is the molarity of the benzethonium chloride solution;

V_3 is the volume, in millilitres, of benzethonium chloride solution used for the titration of a 25-ml aliquot of anionic-active matter solution

Annex H
(normative)

Sampling

H.1 Procedure

H.1.1 In a single consignment, all packages (cartons) containing bathing bars drawn from the same batch of production shall constitute a lot. For ascertaining the conformity of the lot to the requirements of this standard, tests shall be carried out on each lot separately. The number of packages to be selected for drawing the sample shall be in accordance with Table H.1.

Table H.1 — Scale of sampling

Number of packages (cartons) in the lot ***N***	Number of packages (cartons) to be selected ***n***	Number of samples
4 to 15	3	3
16 to 40	4	4
41 to 65	5	2
66 to 110	7	2
111 and above	10	1

H.1.2 The packages shall be selected at random, using tables of random numbers. If these are not available, the following procedure shall be applied:

H.1.3 Starting from any package, count all the packages in one order as 1, 2, 3.... N, selecting every k^{th} package, where k is the integral part of $N \div n$.

H.1.4 From each package thus selected, draw at random an equal number of cakes so as to obtain a total mass of at least 2 kg.

H.2 Preparation of test samples

H.2.1 Composite sample

Weigh each cake separately (including any material that may have adhered to the wrapper), and calculate the average mass. Cut each of the remaining cakes into eight parts by means of three cuts at right angles to each other through the middle. Grate finely the whole of two diagonally opposite eighths of each specimen. Mix the gratings and place in a clean, dry, airtight glass container.

H.2.2 Samples for testing

Immediately after preparation of composite sample (H.2.1), take at one time all test samples required for the tests in 4.2. Weigh out the test sample required for determination of free alkali or acid content, and use it immediately.

Annex I
(informative)

Permitted structuring and processing aids

The following structuring and processing aids are generally used in antibacterial bathing bars:

- Starch and derivatives
- Cellulose and derivatives
- Propylene glycol
- Sorbitol
- Glycerol
- Dextrin
- Kaolin
- Talc
- Bentonite
- Calcite
- Sodium lactate
- Soda ash
- Vegetable/animal oil fatty acids and salts
- Phosphates
- Sodium chloride
- Sodium sulphate
- Dolomite
- Fatty alcohol
- Rosin and rosin salts
- Fatty acid ethanolamide
- Diethylene glycol monostearate
- Paraffin
- Polyoxyethylene glycol
- Glycerol monostearates
- Silicates

- Sodium citrate
- Chelating agents
- Any other internationally accepted builder

Annex J
(informative)

Permitted antibacterial agents

The following antibacterial agents are generally used in antibacterial bathing bars.

a) Triclosan (TCN)

b) Trichlorocarbanilide (TCC)

c) Zinc oxide

d) Hexa chlorophene

e) Chloro xylenols

f) Plant extracts

g) Any other internationally accepted antibacterial agent.