Antibacterial bathing bars — Specification
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Foreword

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Introduction

Performance of soaps, for a long time, 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 antibacterial soap product.

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

1 Scope

This African Standard specifies the requirements and methods of sampling and test for solid antibacterial bathing bars used for personal care.

This Standard applies to antibacterial bathing bars supplied in the form of bars 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/synthetic 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.

ARS 1462, Powder detergents — Biodegradability
ARS 1468, Anti-bacterial liquid toilet soap — Specification
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

3 Terms and definitions

For the purpose of this standard the following definitions apply.

antibacterial bathing bar
the bathing bar is a product containing soap of fatty acids with or without synthetic surface-active agents which contains antibacterial agent(s).

4 Requirements

4.1 General requirements

4.1.1 Antibacterial bathing bars shall be in the form of bars.

4.1.2 The colour of the bar shall generally be uniform, except for multi-coloured bars.

4.1.3 The product shall not be harmful to skin.

4.1.4 The antibacterial bathing bars shall be firm.

4.1.5 They may contain suitable quantities of colouring matter, perfume, opacifiers and optical brightening agents.

4.1.6 Antibacterial bathing bars shall not contain any ingredients in amounts that are harmful to the human body and environment.
4.1.7 The antibacterial bathing bar shall contain permitted antibacterial agent. The label shall clearly state the antibacterial agent used and its level.

4.1.8 The antibacterial bathing bar shall pass the antibacterial activity test when determined by the method given in Annex J.

4.2 Specific requirements

Antibacterial bathing bars shall also comply with the requirements given in Table 1 when tested against the methods prescribed therein.

<table>
<thead>
<tr>
<th>No.</th>
<th>Characteristic</th>
<th>Requirement</th>
<th>Test method</th>
</tr>
</thead>
<tbody>
<tr>
<td>(ii)</td>
<td>Lather, ml, min.</td>
<td>200</td>
<td>Annex A</td>
</tr>
<tr>
<td>(iii)</td>
<td>Mush (loss in mass due to mushing on a wet surface), g/30 cm², max.)</td>
<td>10</td>
<td>Annex B</td>
</tr>
<tr>
<td>(iv)</td>
<td>Freedom from grittiness</td>
<td>Pass test</td>
<td>Annex C</td>
</tr>
<tr>
<td>(v)</td>
<td>Total alkalinity (as NaOH), % by mass, max.</td>
<td>1.0</td>
<td>ISO 685</td>
</tr>
<tr>
<td>(vi)</td>
<td>Free caustic alkali (as NaOH), % by mass, max.</td>
<td>0.3</td>
<td>ISO 456</td>
</tr>
</tbody>
</table>

4.3 Ingredients

The antibacterial bathing bar shall have one or more of the following surfactants:

4.3.1.1 There is no restriction on the use of soap of fatty acids, fatty acid ester sulphonates, fatty alkanamide, fatty alcohol ethoxylates, sarcosinates, taurides, fatty isothionates, alpha olefin sulphonates, alcohol sulphates and amphoteric such as betaines and fatty alcohol ethoxy sulphate, linear alkyl benzene sulphonates (LAS) and alkyl poly glycosides (APG) and fatty alcohol sulphonates and fatty alkanol amido sulphosuccinate or any other safe surfactant.

4.3.1.2 In addition to the surfactants and perfume, the antibacterial bathing bar may contain other Ingredients such as electrolytes, bar structuring and processing aids, colouring matter, permitted antioxidants, preservatives, permissible germicides, super fatting agents, humectants and plant extracts.

4.3.1.3 Rosins, as % of total fatty matter, shall not exceed 2 % m/m when tested against annexes J and K.

4.3.1.4 The synthetic surface-active agents shall pass the biodegradability test as given in ARS 1462.

4.3.2 All ingredients shall be declared on the label.

4.3.3 The bathing bar shall not contain any materials prohibited by applicable regulations.

5 Sampling

5.1 Scale of sampling

The consignment of Antibacterial bathing bar cakes to be sampled shall be divided into lots, each lot containing about 20 cakes from the same source and of the same declared weight and brand.

From each lot, a sample of 9 cakes shall be drawn at random. The cakes of soap thus chosen shall be kept, until tested, in a clean closed container, marked so as to identify the lot from which they have been chosen.
The cakes shall be tested separately for each of the tests mentioned in Table 1.

This scale of sampling will ensure that there are a further 9 samples remaining with which to repeat any of the tests that give results not conforming to the criteria for conformity, or in the event of a dispute over the results.

6 Packaging and labelling

6.1 Packaging

Each bar shall be wrapped and packed in suitable boxes, packages or cartons to avoid contamination or damage during transportation.

6.2 Labelling

Each antibacterial bathing bar shall be marked legibly and indelibly with the following particulars:

a) the words “Antibacterial Bathing Bar”;

b) Product Name

c) manufacturer’s name and physical address and trade mark if any;

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

d) nominal weight of each bar at the time of packaging;

e) number of bars contained in the package;

f) batch number or code number

g) all ingredients;

h) date of manufacture and expiry date;

i) antibacterial agents used and their levels;

j) country of origin, if different from manufacturer address
Annex A
(normative)

Test for lather volume

A.1 General

Strict attention shall be paid to all details of the procedure in order to ensure concordant results. Particular 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 minutes.

A.3 Reagents

A.3.1 Calcium chloride CaCl₂·2H₂O, AR

A.3.2 Magnesium sulphate MgSO₄·7H₂O, AR

A.3.3 Distilled water

A.4 Apparatus

A.4.1 Graduated cylinder — Glass stoppered with graduation from 0 to 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 – 110°C

A.5 Preparation of standard hard water

Dissolve 0.220 g of calcium chloride dihydrate 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 ppm calculated as calcium carbonate.

A.6 Sample preparation

Cut away the outer edges of bathing bar using a knife

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

A.7 Procedure

Weigh 1 g of the grated chips antibacterial bathing bar 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.

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.
Adjust the contents in the cylinder to 100 mL by adding sufficient standard hard water. Bring the contents of the cylinder to 30 °C. Stir the contents of the cylinder with a glass rod or thermometer to ensure a uniform suspension.

As soon as the temperature of the contents of the cylinder reach 30 °C, 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 (V1 mL).

b) water only (V2 mL).

**Figure A.1 — Measurement of foam**

### A.8 Calculation

Lather volume = \( V_1 - V_2 \)

where

\[ V_1 = \text{Volume, in mL of foam + water;} \]

\[ V_2 = \text{Volume, in mL of water only.} \]
Annex B
(normative)

Evaluation of the mushing properties of a bathing bar

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/ 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 cm² 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.2.1.4 Other equipment/ materials for the test

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.

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.

All 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.

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

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.3.2 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

For each test piece
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 e.g. 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 test piece and holder are weighed (W₁).

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

B.4.7 The test piece is reweighed (W₂) and the amount of mush removed is calculated as in D.5. The mush is expressed as grams per 30 cm² 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 soaps 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

Weight of mush (grams) \( W = W₁ − W₂ \)

Surface area of bar (cm²) \( A = \text{width} \times \text{breadth} \)

Mush = \( \frac{W \times 30g}{A} \) per 30 cm²

B.6 Criteria for conformity

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.

The sample lot of products shall be declared as conforming to the requirements for this standard if \( X + 0.6R \) is less than the maximum value given in Table 1.
Annex C
(normative)

Determination of grittiness in antibacterial bathing bar

C.1 Procedure

Either

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.

or

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).

Allow the used bar to dry in the open for 4 hours and examine the surface.

A set of 3 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 3 sets of products (9 grit tests).

NOTE 2 If using a bowl rather than running water use fresh water after testing every set of 3 samples.

C.2 Criteria for conformity

The performance criteria are:

During manipulation under running water the washing bar will not have a visibly rough surface and will feel smooth to the touch. No gritty particles will be observed on the surface of the dried bar 4 h after the washing test.
Annex D  
(informative)

Determination of TCC and TCN in antibacterial bathing bar 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 ppm of the above compounds:

Procedures for both normal and reverse HPLC has 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 Chromatograph 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 5μ (Lichrosorb Si-60)

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

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 flash and make upto 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.2.2.7.1 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

Detector wavelength flow rate: 280 nm

Flow rate: 0.5 ml/min

Injection volume: 20 μl

Retention time

Silica column
TCN - 7.5 min
TCC - 19.2 min

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 ppm).

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 area of the peaks of respective retention time for standard and sample.

D.2.4 Calculation

\[
\text{TCN, % by mass} = \frac{\text{Area of sample for TCN} \times \text{Concentration of standard TCN}}{\text{Area of standard TCN} \times \text{Concentration of sample}} \times 100
\]
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 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.3.2 Apparatus

D.3.2.1 Column

Octyldimethylsilyl (C-DB)
Supercosil LC-8-DB - 15 cm x 4.6 mm, 5 μm

D.3.2.2 Mobile phase

MeOH/0.01 M Phosphate buffer 62:38 v/v

0.01 M Phosphate buffer: Dissolve 1.38 g sodium dihydrogen phosphate monohydrate in 1 000 ml of distilled water. Prepare to pH 3.0 by 10 % phosphate solutions.

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 of 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 (D.3.3.1.1) into the (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

Detector wavelength 280 nm
Column temperature 35 ºC
Flow rate 1.0 ml/min
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 μg the sample solution and determine each ingredients percentage by the calculation shown.

D.3.4 Calculations

\[
\text{TCN, % by mass} = \frac{M_t \times A_r \times F}{A_s \times M_s \times 100}
\]

\[
\text{TCC, % by mass} = \frac{M_t \times A_r \times F}{A_s \times M_s \times 100}
\]

where

- \( A_r \) is the peak area of the test sample,
- \( A_s \) is the averaged peak area of the standard,
- \( F \) is the purity of standard (percent).
- \( M_s \) is the mass, in grams, of the standard, and
- \( M_t \) is the mass, in grams, of the test sample,

NOTE Both TCC and TCN are photosensitive, hence standards should be freshly prepared.
Annex E
(ininformative)

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 percent 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 — 100 micron mesh.

E.3.8 Solvent mixture

DMSO 5 volumes
n-Butanol 2 volumes
distilled water 2 volumes
hydrochloric acid 1 volume

Mix n-butanol, water and HCL, cool the mixture and add DMSO.

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 Stop watch

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 0.3498 g of 3, 4-dichloroaniline and 0.2753 g of 4-chloroaniline in solvent mixture (see C.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 solvent mixture
   1 ml = 50 μg mixed chloroanilines.

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, 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 °C 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 C.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 In preparing the blank solution, take 40 ml of solvent mixture in a 50 ml amber volumetric flask. Incubate the flask in a water bath at 25 °C 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 (swirl the flask occasionally). Then add 2 ml of reagent (see E.3.4) into the flask and return it to the water bath for exactly 10 min. 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 μg of chloroanilines in all different aliquots of standard.

E.6 Determination of chloroanilines

E.6.1 Weigh to the nearest mg 3.0 - 15 g of finely grated soap 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 a 50 ml amber volumetric flask. Add 20 ml of solvent mixture. The flask is incubated in a water bath at 25 °C 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 stop watch). 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. 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 °C 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 this solution as a blank for reading sample only.

E.6.3 Deduce the amount of chloroanilines (μg) from the calibration graph curve.

NOTE The determination should be completed in one day.

E.7 Calculations

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

\[
\text{Chloroaniline content (in ppm)} = \frac{250(M + M_1)M_3}{20M_1M} 
\]

where

- \(M\) is the mass, in grams, of soap
- \(M_1\) is the mass, in grams, of sand
- \(M_2\) is the mass, in grams, of soap and sand transferred to the flask
- \(M_3\) is the mass, in micrograms (μg) of mixed chloroanilines found from calibration graph/or it can be calculated as given below:

\[
M_3 = \frac{\text{Mass of the sample}}{\text{Average absorbance of 1 μg mixed chloroanilene (AA)}}
\]

where

\[
M_3 = \frac{\text{Sum of the OD of the standards}}{\text{Sum of concentration of standard chloroanilines in μg}}
\]

Weight of soap actually used, in g = \(\frac{M_2M}{(M + M_1)}\)
Annex F
(informative)

Permitted structuring and processing aids

The following is the list of structuring and processing aids used generally in bathing bars.

1) Starch and derivatives
2) Cellulose and derivatives
3) Propylene glycol
4) Sorbitol
5) Glycerol
6) Dextrin
7) Kaolin
8) Talc
9) Bentonite
10) Calcite
11) Sodium lactate
12) Soda ash
13) Vegetable/animal oil fatty acids and salts
14) Phosphates
15) Sodium chloride
16) Sodium sulphate
17) Dolomite
18) Fatty alcohol
19) Rosin and rosin salts
20) Fatty acid ethanolamide
21) Diethylene glycol monostearate
22) Paraffin
23) Polyoxyethylene glycol
24) Glycerol monostearates
25) Silicates
26) Sodium citrate
27) Chelating agents
28) Any other internationally accepted builder
Annex G
(informative)

Permitted antibacterial agents

The following is the list of antibacterial agents used generally 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.
Annex H
(normative)

Determination of microbial inhibition of cosmetic soap bars and liquid hand and body washes

H.1 Scope
This annex specifies a method for testing and comparing the microbial inhibition properties of cosmetic soap bars and liquid hand and body washes.

H.2 Application
This method applies to the verification of efficacy claimed by antibacterial soaps in the inhibition of microorganisms.

H.3 Principle
When soap bars and liquid hand and body washes that have antimicrobial properties are inoculated into medium No.1 (see J.4.3.1.1), specific active ingredients diffuse into the surrounding agar thereby creating a zone of inhibition around the product, which is measured as an indication of the microbial inhibition properties of the product.

NOTE The organisms referenced in this standard are the indicator organisms used when testing for hygiene purposes.

H.4 Test method
H.4.1 General
Sampling and testing shall be carried out by personnel familiar with microbiological procedures.

H.4.2 Accuracy
Except where otherwise specified, all the following tolerances:
- temperatures ± 2 °C;
- masses ± 1.0 %;
- volumes ± 1.0 %; and
- pH values ± 0.1 pH unit.

H.4.3 Culture medium, reagents, reference cultures and controls

H.4.3.1 Culture medium

NOTE 1 The culture medium listed in J.4.3.1.1 is commercially available in dehydrated form and is made up in accordance with the manufacturer’s instructions.

NOTE 2 Glass distilled water or deionised water should be used

H.4.3.1.1 Medium No. 1

Ingredients

- agar 15.0 g
- beef extract 15.0 g
- glucose 1.0 g
- pancreatic digest of casein 4.0 g
- peptone 6.0 g
- Yeast extract 3.0 g
- Water 1 000 mL
H.4.3.1.2 Preparation

H.4.3.1.2.1 Dissolve the ingredients in approximately 900 mL of water and mix.

H.4.3.1.2.2 Make up to 1 L.

H.4.3.1.2.3 Adjust the pH so that after sterilization the pH value is 6.6 ± 0.05.

H.4.3.1.2.4 Dispense 20 mL ± 0.2 mL volumes into suitable containers and sterilize in an autoclave for 15 min ± 0.5 min at 121 °C.

H.4.3.2 Horse serum

Sterile inactivated horse serum that is free from preservatives.

H.4.3.3 Reference cultures

H.4.3.3.1 Test organisms

Use the following test organisms:

- **Staphylococcus aureus** Sta 10 ATCC 6538;
- **Escherichia coli** Esc 20 ATCC 8739; and
- **Pseudomonas aeruginosa** Pse 16 ATCC 15442.

**NOTE** Other reference organisms may also be used in addition to the ones specified in J.4.3.3.1.

H.4.3.3.2 Preparation of test organism suspensions

From a newly opened freeze-dried culture or recently received agar culture, subculture the test organisms into bottles of 10 mL nutrient medium (J.4.3.3.2.1)

H.4.3.3.2.1 Nutrient medium

H.4.3.3.2.1.1 Ingredients

- peptone 5.0 g
- sodium chloride 5.0 g
- yeast extract 2.0 g
- beef extract 1.0 g
- water 1 000 mL

H.4.3.3.2.1.2 Preparation

Dissolve the ingredients in the water and adjust the pH value to 7.1. Dispense in 10 mL volumes into suitable bottles and sterilize by autoclaving at 121 ±2 °C for 15 minutes.

H.4.3.3.3 Incubate the bottles at 37 °C for 24 h. Subculture onto nutrient agar slopes (see J.4.3.3.3.1). Incubate the slopes at 37 °C for 24 h.

H.4.3.3.3.1 Nutrient agar

H.4.3.3.3.1.1 Ingredients

- Agar 15.0 g
- peptone 5.0 g
- sodium chloride 5.0 g
- yeast extract 2.0 g
- beef extract 1.0 g
- water 1 000 mL
H.4.3.3.3.2 Preparation

Dissolve the ingredients in the water and adjust the pH value to 7.1. Dispense 10 mL and 15 mL volumes into suitable bottles and sterilize by autoclaving at 121 ±2 °C for 15 minutes. Allow only the 10 mL volumes to solidify in a sloped position.

H.4.3.3.4 From each of these slope cultures, prepare four subcultures (stock cultures) of each test organism onto 10 mL nutrient agar slopes (see J.4.3.3.1). Incubate the stock cultures at 37 °C for 24 h and then store in a refrigerator maintained at 4 °C, except for *Pseudomonas aeruginosa* which is stored at ambient temperature.

H.4.3.3.5 Use the stock cultures to prepare further subcultures for the test, but do not make more than six serial subcultures from each stock culture. After the sixth serial subculture, resort to a new freeze-dried culture.

H.4.3.3.6 Preparation of cultures for test suspensions

H.4.3.3.6.1 For each of the test organisms, inoculate a nutrient agar slope (see J.4.3.3.1) from a stock culture (see J.4.3.3.4) and incubate at 37 °C for 24 h.

H.4.3.3.6.2 For the test, use a 24h culture that has been subcultured for two successive days. After six subcultures, restart the process using a fresh stock culture (see J.4.3.3.4).

**NOTE** The physiological condition of the test organisms is important and might influence inter-laboratory and intra-laboratory variations in test results.

H.4.3.3.6.3 After incubation, wash the bacterial growth from the slope using 10 mL sterile water and, if necessary, scrape the agar surface. Carefully decant the suspension into a sterile Erlenmeyer flask and shake vigorously to suspend all growth in the water. Standardize the suspension, by using a spectrophotometer in conjunction with a standard curve, a haemocytometer, Petroff-Hausser counting chamber or any other suitable means, so that it contains $10^5$ cfu/mL ± $10^4$ cfu/mL. Use the suspension within 3 h of preparation.

H.4.3.4 Control

H.4.3.4.1 Positive control

Natural honey with no additives, e.g. colourants

H.4.3.4.2 Negative control

Sterile deionised water.

H.4.4 Procedure

H.4.4.1 Melt the contents of a bottle of the medium No. 1 (see J.4.3.1.1) and cool to 45 ± 2 °C. Add 1 mL of the sterile inactivated horse serum (see J.4.3.2) and 1 mL of S. aureus test organism suspension prepared in accordance with J.4.3.3.2. Mix well and avoid the formation of air bubbles, then pour the mixture into a Petri dish of 90 mm diameter and allow to solidify.

H.4.4.2 Using as sterile cutter that produces cylindrical wells (holes) of 8 mm diameter, make five evenly spaced straight cylindrical wells in the solidified medium. Remove and discard the plugs of medium. Seal the bottom of each well using one or two drops of the molten medium in J.4.3.1.1 and allow to solidify.

H.4.4.3 Introduce the test sample into the wells in such a way that each well is completely filled with the sample, taking care not to form bubbles. When the test sample is a soap bar, grind a sufficient amount to a powder, using a sterile pestle and mortar, and introduce the powder into the wells. Ensure that the surface of the agar remains free from the sample.

H.4.4.4 Incubate the petri dish at 37 ± 1 °C for 18 h to 24 h.
H.4.4.5 At the end of this period (see J.4.4.4), remove the Petri dish from the incubator. Measure the
diameter of the zone of inhibition diagonally across the well to the nearest millimeter.

H.4.4.6 Take the average of the five diameters and record this to the nearest millimeters.

H.4.4.7 Repeat the procedure described in J.4.4.1 to J.4.4.6 (inclusive) using the *E. coli* and *P. aeruginosa* test suspensions successively.

H.4.4.8 Repeat the procedure described in J.4.4.1 to J.4.4.6 (inclusive) for the positive control and the negative control.

H.5 Interpretation of results

H.5.1 For the product to pass the test, the average diameter of the zone of inhibition for each of the test organisms shall be at least 10 mm.

H.5.2 If the zones are not clearly defined for one of the reference organism (e.g., hazy, incomplete zone, or distorted shape), the test shall be repeated for the specified reference organism.
Annex J
(normative)

Identification of rosin content of fatty matter in soaps

J.1 Scope
This Annex specifies the Halphen-Grimaldi and Liebermann-Storch method for the identification of rosin in fatty matter.

J.2 Reagents
NOTE Use only analytical grade reagents and distilled water.

J.2.1 Acetic anhydride.

J.2.2 Sulphuric acid, density 1.53 g/mL. Cautiously add 97 mL of sulphuric acid (density 1.84 g/mL) to 100 mL of water.

J.2.3 Solution A. Dissolve 10 g of phenol in 27 mL of chloroform.

J.2.4 Solution B. Dissolve 10 mL of bromine in 40 mL of chloroform.

J.3 Procedure

J.3.1 Halphen-Grimaldi method

J.3.1.1 Place one or two drops of the fatty matter, reserved from the determination of fatty matter (ISO 685), in a porcelain basin and add approximately 2 mL of the solution A to dissolve it. Wet the walls of the basin by tilting and turning it and let it stand for a few seconds so that the walls of the basin are covered with a very thin film of the solution.

J.3.1.2 Hold the neck of the flask containing the solution B in such a position that the bromine vapour diffuses into the porcelain dish and comes into contact with the walls. In the presence of rosin a blueish-grey to purple colour develops immediately.

J.3.1.3 Carry out a comparison test with a sample of fatty matter to which 2 % (m/m) of rosin has been added.

NOTE Should the colour reaction in J.3.1.2 be masked by other colours, conduct a second qualitative test, using the Liebermann-Storch method.

J.3.2 Liebermann-Storch method

J.3.2.1 Place 1 g - 2 g of the fatty matter in a test tube, add 5 mL - 10 mL of the acetic anhydride, and heat the mixture in a boiling water bath for approximately 3 min. Cool to room temperature and pour 1 mL - 2 mL of the solution into a white porcelain basin.

J.3.2.2 Allow one or two drops of the sulphuric acid to run down the side of the basin. If rosin is present, a violet colour immediately develops where the acid is in contact with the solution. This colour then turns brown on standing.

J.3.2.3 Carry out a comparison test with a sample of fatty matter to which 2 % (m/m) of rosin has been added.
Annex K
(normative)

Determination of rosin content of fatty matter in soaps

K.1 Scope

This Annex specifies a method for the determination of the rosin content of fatty matter in soaps.

NOTE 1 Before proceeding with the quantitative determination of rosin, first establish its presence by a qualitative test (see Annex J).

NOTE 2 The following method is not accurate for rosin concentrations below a mass fraction of 5 %.

K.2 Reagents

NOTE Use only analytical grade reagents and distilled water.

K.2.1 Diethyl ether

Free from peroxides.

K.2.2 Sodium chloride

K.2.3 Sodium sulfate

Anhydrous.

K.2.4 Hydrochloric acid solution

Dilute one volume of concentrated hydrochloric acid (density 1.16 g/mL) with two volumes of distilled water.

K.2.5 Sodium hydroxide solution

10 % mass fraction aqueous solution.

K.2.6 Naphthalene-2-sulfonic acid solution

Dissolve 40 g of naphthalene-2-sulfonic acid in 1 L of absolute methanol.

K.2.7 Sodium chloride solution, saturated

Shake an excess of sodium chloride with water at ambient temperature until no more dissolves. Keep the solution over solid sodium chloride.

K.2.8 Sodium chloride solution, 10 %

Dissolve 10 g of sodium chloride in 100 mL of water.

K.2.9 Standard ethanolic potassium hydroxide solution, 0.2 N

K.2.9.1 Preparation

Purify 95 % (by volume fraction) ethanol by boiling 1.5 L of it over 20 g of potassium hydroxide for 1 h under reflux. Distil, discarding the first 50 mL of the distillate and stopping the distillation when approximately 1.3 L have been distilled. Dissolve 12 g of potassium hydroxide in 1 L of the purified ethanol, allow the solution to stand for approximately one week, and then decant the clear supernatant liquid from any potassium carbonate that has precipitated.
K.2.9.2 Standardization

Accurately weigh out approximately 1 g of potassium hydrogen phthalate (previously dried at 110 °C ± 5 °C for 3 h) into a 250 mL Erlenmeyer flask. Add approximately 100 mL of carbon dioxide-free distilled water and three drops to five drops of the phenolphthalein indicator, and swirl gently until the solid has dissolved. Titrate the solution with the standard ethanolic potassium hydroxide solution, until a permanent pink colour is formed.

K.2.9.3 Calculation

Normality of the standard ethanolic potassium hydroxide solution ($N$):

$$N = \frac{A \times 4.897}{B}$$

where

$A$ is the mass of the potassium hydrogen phthalate, in grams;

$B$ is the volume of the standard ethanolic potassium hydroxide solution used for the titration, in millilitres.

K.2.10 Methyl orange indicator

Dissolve 0.2 g of methyl orange in 100 mL of carbon dioxide-free water.

K.2.11 Phenolphthalein indicator

Dissolve 0.5 g of phenolphthalein in 100 mL of freshly boiled, 95 % (by volume fraction) ethanol.

K.3 Procedure

K.3.1 Preparation of fatty matter

K.3.1.1 Carbolic soaps

K.3.1.1.1 Weigh out accurately into a 600 mL beaker such quantity of the test sample as contains approximately 40 g of fatty matter and dissolve it in approximately 400 mL of hot water, to which 40 mL of the sodium hydroxide solution has been added.

K.3.1.1.2 Salt out the soap by adding sufficient sodium chloride to the hot solution to saturate it (at ambient temperature) with sodium chloride. Filter the soap quantitatively, and allow it to drain.

K.3.1.1.3 Dissolve the drained soap in approximately 400 mL of hot water, and repeat the salting out and filtering procedure.

K.3.1.1.4 Wash the soap thoroughly with the saturated sodium chloride solution, and proceed in accordance with K.3.1.2.2 to K.3.1.2.7.

K.3.1.1.5 Reserve the combined filtrates and washings for the determination of carbolic acids.

K.3.1.2 Other soaps

K.3.1.2.1 Weigh accurately into a 600 mL beaker such quantity of the test sample as contains approximately 40 g of fatty matter.

K.3.1.2.2 Dissolve the soap in approximately 400 mL of hot distilled water, cool the solution, and slowly add an excess of the hydrochloric acid solution. Cover the beaker with a watch-glass and heat the contents until the fatty matter separates into a clear layer, but do not allow the temperature to exceed 60 °C.
K.3.1.2.3 In the cases of liquid and gel soaps, acidify the soap as it is.

K.3.1.2.4 Cool to approximately 25 °C and transfer the contents of the beaker to a separating funnel. Rinse the watch-glass and the beaker with portions of the diethyl ether totalling 100 mL and add them to the separating funnel. Shake the mixture in the separating funnel vigorously for 1 min, and let it stand until the two phases have separated.

K.3.1.2.5 Draw off and discard the aqueous layer and wash the ether extract with 50 mL portions of the sodium chloride solution until the last washing is neutral to the methyl orange indicator.

K.3.1.2.6 Filter the washed ether extract into a 250 mL beaker through a filter paper containing approximately 5 g of anhydrous sodium sulfate, and wash the separating funnel and the filter with small portions of the diethyl ether.

K.3.1.2.7 Evaporate the ether extract plus washings on a warm water bath. When the residue is just dry, heat it rapidly to 130 °C and immediately place the beaker in a desiccator to cool.

NOTE K.3.1.2.2 to K.3.1.2.7 should be completed in the shortest possible period of time, so as to prevent oxidation of the fatty acids.

K.3.2 Determination of rosin content

K.3.2.1 Weigh accurately into a 150 mL flask with a ground-glass joint approximately 2 g of the prepared fatty matter (K.3.1.2.7) and reserve the rest in the case of soap powders for the fatty matter titre determination.

K.3.2.2 Add exactly 25 mL of the naphthalene-2-sulfonic acid solution to the flask and two or three glass beads, and boil under reflux for 30 min.

K.3.2.3 Carry out a blank test at the same time, using exactly 25 mL of the naphthalene-2-sulfonic acid solution only.

K.3.2.4 Cool the contents of both flasks (sample and blank) to ambient temperature, add 0.5 mL of the phenolphthalein indicator to each flask, and immediately titrate to the end point with the standard ethanolic potassium hydroxide solution.

K.4 Calculation

Rosin content of fatty matter, as a mass fraction percentage (R):

\[ R = \left(\frac{A - B}{C}\right) \times N \times 34.6 - 1.0 \]

where

A is the volume of the standard ethanolic potassium hydroxide solution used for the titration of the sample solution, in millilitres;

B is the volume of the standard ethanolic potassium hydroxide solution used for the titration of the blank solution, in millilitres;

N is the normality of the standard ethanolic potassium hydroxide solution;

C is the mass of the fatty matter taken, in grams.
Bibliography

EAS 794:2013, Kenya Standard — Determination of the microbial inhibition of cosmetic soap bars and liquid hand and body washes — Test method

EAS 878:2016, Antibacterial bathing bars — Specification