# AFRICAN TANDARD

# DARS 1468: 2026

First Edition 2026

## Anti-bacterial liquid toilet soap — Specification



Reference No. CD-ARS 1468:2026(E)
ICS 71.100.40

## Table of contents

1 Scope ..... 1
2 Normative references ..... 1
3 Definitions and abbreviations ..... 1
4 Requirements ..... 1
5 Environmental precautions ..... 2
6 Packing and marking ..... 3
6.1 Packing ..... 3
6.2 Marking ..... 3
7 Sampling ..... 3
7.1 Preparation of test samples ..... 3
7.2 Number of tests ..... 3
7.3 Criteria for conformity ..... 3
Annex A (informative) Permitted antibacterial agents ..... **Error! Bookmark not defined.**
Annex B (normative) Determination of TCC and TCN in soaps by HPLC ..... 5
Annex C (normative) Determination of chloroaniline ..... 9
Annex D (normative) Determination of microbial inhibition of cosmetic soap bars and liquid hand and body washes ..... 12
Bibliography ..... 18

## Foreword

The African Organization for Standardization (ARSO) is an African intergovernmental organization established by the United Nations Economic Commission for Africa (UNECA) and the Organization of African Unity (AU) in 1977. One of the fundamental mandates of ARSO is to develop and harmonize African Standards (ARS) for the purpose of enhancing Africa's internal trading capacity, increase Africa's product and service competitiveness globally and uplift the welfare of African communities. The work of preparing African Standards is normally carried out through ARSO technical committees. Each Member State interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, Regional Economic Communities (RECs), governmental and non-governmental organizations, in liaison with ARSO, also take part in the work.

ARSO Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare ARSO Standards. Draft ARSO Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an ARSO Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ARSO shall not be held responsible for identifying any or all such patent rights.

This African Standard was prepared by the ARSO Technical Committee Number 35 on Surface Active Agents (ARSO/TC 35).





ARSO Central Secretariat
International House 3rd Floor
P. O. Box 57363 — 00200 City Square
NAIROBI, KENYA

Tel. +254-20-2224561, +254-20-3311641, +254-20-3311608
Fax: +254-20-218792
E-mail: arso@arso-oran.org
Web: www.arso-oran.org

ARSO Central Secretariat
International House 3rd Floor
P.O. Box 57363 — 00200 City Square
NAIROBI, KENYA

Tel: +254-20-2224561, +254-20-3311641, +254-20-3311608
Fax: +254-20-218792

E-mail: arso@arso-oran.org
Web: www.arso-oran.org

## Introduction

Human skin provides a favourable environment for the existence and multiplication of a variety of microbes. The conventional toilet soap washes away the germs but does not kill them. The function of an antibacterial or antiseptic toilet soap is not only to clean the skin, but also to reduce drastically the bacterial count on the skin. This prevents skin infections and perspiration odour caused by the decomposition of perspiration by bacteria.

Antibacterial toilet soap is a toilet soap that has antibacterial agents incorporated into it. It not only cleans the skin, but also reduces drastically the bacterial count on the skin. This prevents skin infections and perspiration odour caused by the decomposition of sweat by bacteria. The antibacterial toilet soap is specially effective against *staphylococcus* and similar bacteria which have the habit of residing in the under layers of the skin. The antibacterial soaps have to be used regularly to be effective.

In this revision, hexachlorophene has not been permitted to be used as antibacterial agent. The Trichlorocarbanilide (TCC) on heating decomposes to chloroanalines which are harmful to skin hence the limit and method for determination of chloroanaline is included. Use of other antibacterial agents not included in Annex A will be considered when need arises as long as their safety is assured.

# AFRICAN STANDARD

# Anti-bacterial liquid toilet soap — Specification

## 1 Scope

This Draft African Standard specifies the requirements, sampling and methods of test for antibacterial liquid toilet soap. It includes antibacterial (bacteriostatic) and antifungal (fungal static).

The standard does not cover synthetic hand wash liquid detergents, shampoo and products for specific purposes such as those for industrial and surgical uses.

## 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.

DARS 496-2:2017, *Synthetic detergent powders — Part 2: Composition-based household hand wash*

DARS 1524-1 *Cosmetics — Safety of cosmetic products ingredients — Part 1: List of prohibited substances*

*DARS 1524-2 Cosmetics — Safety of cosmetic products ingredients — Part 2: List of substances which cosmetic products must not contain except subject to the restrictions laid down*

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 673, *Analysis of soap — Determination of matter insoluble in ethanol*

ISO 862, *Surface active agents — Vocabulary*

ISO 8212, Soaps and detergents — Techniques of sampling during manufacture

## 3 Definitions and abbreviations

For the purpose of this standard the terms and definitions given in ISO 862 and the following shall apply.

ISO and IEC maintain terminological datatbases for use in standards at the following addresses
- IEC Electropedia: available at http:/.www.electropedia.org/
- -ISO online browsing platform: available at http://www.iso.org/obp

## 4 Requirements

### 4.1 General Requirement

**4.1.1** The antibacterial soap shall consist of essentially of an aqueous solution of potassium soaps, sodium soaps or both, made from oils, fatty acids or their mixture. It shall be a homogeneous, clear, translucent or opaque liquid with good lathering and cleaning properties. It may contain permissible synthetic detergents.

**4.1.2** The antibacterial soap shall remain as homogeneous stable product and shall show no sign of separation or sedimentation when kept at 5 °C for 24h.

**4.1.3** The soap shall contain permitted antibacterial agent as specified in DARS 1524-1 and DARS 1524-2.

**4.6** The antibacterial toilet soap shall not contain any material listed as a prohibited in DARS 1524-1 and DARS 1524-2.

### 4.2 Specific requirements

Antibacterial liquid toilet soap shall also comply with the specific requirements specified in Table 1.

**Table 1 — Specific requirement for antibacterial liquid toilet soap**

| | Characteristic | Requirement | Method of test |
|---|---|---|---|
| 1 | Total fatty matter, percent by mass, min | 15.0 | ISO 685 |
| 2 | Free caustic alkali, ($K_2O$), % by mass, max. | 0.03 | ISO 456 |
| 3 | Synthetic detergents, percent by mass, max. | 2.0 | DARS 496-2:2017 |
| 4 | Matter insoluble in water, percent by mass, max | 0.5 | |
| 5 | Antibacterial agent Triclosan (TCN) and Trichlorocarbanilide (TCC), percent by mass, max<br>TCN percent by mass, max<br>TCC percent by mass, max | 1,0 ( in combination)<br>0,3 (singly)<br>1.5 (singly) | Annex A |
| 6 | Chloroaniline content, ppm, max | 10 | Annex B |
| 7 | Phosphate | 0 | DARS 496-2:2017 |
| | Antibacterial activity | Shall pass test | Annex C |
| | Irritation to product | shall not be irritating to normal skin | Annex D |
| | Heavy Metals<br>Lead, mg/kg, max<br>Arsenic, mg/kg, max<br>Mercury, mg/kg, max | <br>10<br>2<br>1 | ISO 21392 |

NOTE 1 - The total amount of heavy metals as lead, mercury and arsenic, in combination, in the finished product should not exceed 10 mg/kg.

NOTE 2 - The heavy metals including lead, mercury and arsenic may be as a result of contamination during processing and should not be deliberately added as ingredients.

NOTE 3 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 Environmental precautions

Environmental precaution shall be adhered to during production and disposal as per the provisions of the relevant national legislations on prevention and control of pollution of water and air.



 

# 6 Packing and marking

## 6.1 Packing

The antibacterial soap shall be packed in protective containers that will not allow for damage of the product or its contamination.

## 6.2 Marking

Each container shall be marked legibly and indelibly with the following particulars:

a) name and address of manufacture, supplier or importer and/or trade mark if any

b) name of product “anti-bacterial liquid toilet soap”

c) net weight of each container;

d) batch number or lot number;

e) date of manufacture and best before date;

f) list of ingredients

g) antibacterial agents used and their levels;

h) country of origin

# 7 Sampling

## 7.1 Preparation of test samples

For this purpose, scale of sampling and preparation of test samples shall be as prescribed in ISO 8212..

## 7.2 Number of tests

**7.2.1** Tests for determination of total fatty matter and free caustic alkali and matter insoluble in alcohol shall be conducted on each of the individual samples separately.

**7.2.2** Tests for determination of all the remaining characteristics shall be conducted on the composite sample.

## 7.3 Criteria for conformity

**7.3.1** For each of the characteristics which has been determined on the individual samples (see 7.2.1) the mean $(\bar{X})$ and the range *(R)* of the test results shall be calculated as follows:

Mean $(\bar{X})$ = sum of test result/number of test result

Range *(R)* = the difference between the maximum and the minimum value of test results. The lot shall be deemed as conforming to the requirements given in 7.2.1 if the expression $(\bar{X} - 0.6R)$ is greater than or equal to minimum value given in Table 1 and $(\bar{X} + 0.6R)$ is less than or equal to maximum value given in Table 1.

**7.3.2** For declaring the conformity of a lot to the requirements of other characteristics determined on the composite sample, the test results for each of the characteristics shall satisfy the relevant

requirement.

# Annex A
# (normative)

## Determination of TCC and TCN in soaps by HPLC

### A.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.

### A.2 Normal phase HPLC

#### A.2.1 Reagents

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

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

**A.2.1.3 Hexane**, HPLC grade.

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

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

#### A.2.2 Apparatus

**A.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.

**A.2.2.2 Standard volumetric flasks**

**A.2.2.3 Pipettes**

**A.2.2.4 Magnetic stirrer**

**A.2.2.5 Millipore filter apparatus** with 0.5 µ filter

**A.2.2.6 Column**

**A.2.2.6.1 Silica column**, stainless steel 25 cm x 0.46 cm packed with Normal phase-silica 5µ (Lichrosorb Si-60)

**A.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.

**A.2.2.7 Mobile phase**

**A.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.

**A.2.2.7.2** For cyano column — Transfer 50 ml of HPLC grade iso-propanol (2-propanol) into a 500 ml volumetric flask, fill upto the mark with hexane and mix well assemble milllipore filter apparatus and filter the solvent system prior to use.

**A.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.

**A.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.

**A.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

**A.2.3 Procedure**

**A.2.3.1 Standard preparation (see note under A.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).

**A.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.

**A.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.

**A.2.4 Calculation**

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



 

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

**A.3 Reverse phase**

**A.3.1 Reagents**

**A.3.1.1** *Methanol* — HPLC grade.

**A.3.1.2** *Sodium Dihydrogen Phosphate Monohydrate* — Chemical grade.

**A.3.1.3** Standard TCC

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

**A.3 Reverse phase**

**A.3.1 Reagents**

**A.3.1.1 Methanol**, HPLC grade.

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

**A.3.1.3 Standard TCC**

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

**A.3.2 Apparatus**

**A.3.2.1 Column**

Octyldimethylsilyl (C-DB)

Supercosil LC-8-DB - 15 cm x 4.6 mm. 5µ

**A.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.

**A.3.3 Procedure**

**A.3.3.1 Standard preparation** (see Note under A.3.4)

**A.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.

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

**A.3.3.1.3** Accurately pipette 10 ml of the solution prepared in (A.3.3.1.1) into the (A.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.

**A.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.

### A.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.

### A.3.4 Calculations

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

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

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 B
(normative)

## Determination of chloroaniline

### B.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.

### B.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.

### B.3 Reagents

**B.3.1 Dimethyl Sulphoxide(DMSO)**, AR grade.

**B.3.2 Hydrochloric Acid – Concentrated**(specific gravity — 1.18).

**B.3.3 Sodium Nitrite** – 0.4 percent w/v analytical grade, freshly prepared (aqueous).

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

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

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

**B-3.7 Sand,** acid purified 40 — 100 micron mesh.

**B.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.

**B.3.9 4-Chroroaniline and 3, 4-Dichloroaniline,** AR grade.

### B.4 Apparatus

**B.4.1 Spectrophotometer,** suitable for use at 554 nm

**B.4.2 Cuvettes** — Glass (matched pair) 10 mm

**B.4.3 Water bath** — Thermostatically controlled at 25 °C

**B.4.4 Stop watch**

**B.4.5 Standard laboratory glassware**

**B.4.6 Filter Paper,** Whatman No. 541

**B.5 Procedure**

**B.5.1 Preparation of Calibration Curve**

**B.5.1.1** Dissolve 0.349 8 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).

**B.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 B.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.

**B.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 C.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 B.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 B.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 B.5.1.4.

**B.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 B.3.3) into the flask and return it to the water bath for exactly 10 min. Then add 2 ml of reagent (see B.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 B.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.

**B.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.

**B.6 Determination of chloroanilines**



 

**B.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 B.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 B.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 B.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).

**B.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 B.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 B.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.

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

NOTE The determination should be completed in one day.

## B.7 Calculations

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

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

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:

$$M_3 = \frac{\text{Mass of the sample}}{\text{Average absorbance of 1}\mu\text{g mixed chloroaniline (AA)}}$$

where

$$M_3 = \frac{\text{Sum of the OD of the standards}}{\text{Sum of concentration of standard chloroanilines in }\mu\text{g}}$$

$$\text{Weight of soap actually used, in g} = \frac{M_2M}{(M + M_1)}$$

# Annex C
# (normative)

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

## C.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.

## C.2 Application

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

## C.3 Principle

When soap bars and liquid hand and body washes that have antimicrobial properties are inoculated into medium No.1 (see D.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.

## C.4 Test method

### C.4.1 General

Sampling and testing shall be carried out by personnel familiar with microbiological procedures.

### C.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. |

### C.4.3 Culture medium, reagents, reference cultures and controls

#### C.4.3.1 Culture medium

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

NOTE 2 Class distilled water or deionised water should be used

#### C.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 |

#### C.4.3.1.2 Preparation

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

**C.4.3.1.2.2** Make up to 1 L.

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

**C.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.

### C.4.3.2 Horse serum

Sterile inactivated horse serum that is free from preservatives.

### C.4.3.3 Reference cultures

#### C.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 C.4.3.3.1.

#### C.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 (C.4.3.3.2.1)

#### C.4.3.3.2.1 Nutrient medium

##### C.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 |

##### C.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.

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

#### C.4.3.3.3.1 Nutrient agar

##### C.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 |

##### C.4.3.3.3.1.2 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.

**C.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 C.4.3.3.3.1). 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.

**C.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 n new freeze-dried culture.

**C.4.3.3.6 Preparation of cultures for test suspensions**

**C.4.3.3.6.1** For each of the test organisms, inoculate a nutrient agar slope (see C.4.3.3.3.1) from a stock culture (see C.4.3.3.4) and incubate at 37 °C for 24 h.

**C.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 C.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.

**C.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.

**C.4.3.4 Control**

**C.4.3.4.1 Positive control**

Natural honey with no additives, e.g. colourants

**C.4.3.4.2 Negative control**

Sterile deionised water.

**C.4.4 Procedure**

**C.4.4.1** Melt the contents of a bottle of the medium No. 1 (see C.4.3.1.1) and cool to 45 ± 2 °C. Add 1 mL of the sterile inactivated horse serum (see D.4.3.2) and 1 mL of S. aureus test organism suspension prepared in accordance with C.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.

**C.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 C.4.3.1.1 and allow to solidify.

**D.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.

**D.4.4.4** Incubate the petri dish at 37 ± 1 °C for 18 h to 24 h.



 

**C.4.4.5** At the end of this period (see C.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

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

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

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

## C.5 Interpretation of results

**C.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.

**C.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 D
(normative)

## Testing Procedure for Assessment of Skin Irritation Potential in Human Volunteers

### D.1 Objective

To assess the skin tolerance and irritation potential of cosmetic ingredients under controlled conditions using a stepwise human volunteer testing approach.

### D.2 Pre-Study Requirements

Before initiation of the study:

i. Conduct a full safety assessment by a qualified safety assessor.
ii. Review chemical structure, toxicological data, and related substances.
iii. Confirm that no significant health risk to volunteers is foreseeable.
iv. Ensure compliance with ethical requirements and obtain approval where applicable.
v. Define study objectives, methodology, evaluation criteria, and adverse event management procedures.
vi. Obtain written informed consent from all participants.

### D.3 Selection of Volunteers

**D.3.1** Inclusion Criteria

i. Healthy informed volunteers meeting study requirements.
ii. Appropriate age, gender, and skin condition.
iii. Agreement to comply with study instructions.

**D.3.2** Exclusion Criteria

i. Pregnancy or nursing.
ii. Skin damage, tattoos, scars, or active skin disease at test sites.
iii. Medication or medical history affecting skin response.
iv. Participation in conflicting or recent studies.

**D.3.3** Withdrawal Criteria

Participants shall be withdrawn if:

i. Study instructions are not followed;
ii. Illness or adverse reactions occur;
iii. The participant chooses to withdraw.

### D.4 Test Materials

i. Prepare test materials at concentrations appropriate for the study objective and expected exposure conditions.
ii. Use suitable vehicles that do not independently cause irritation.
iii. Include reference/control materials where appropriate.

### D.5 Test Methods

Testing shall follow a stepwise progression from minimal to increased exposure until the study objective is achieved.

**D.5.1** Examples of Test Types

**D.5.1.1** Single Application Open Test
Apply material to exposed skin under non-occlusive conditions.

**D.5.1.2** Repeat Application Open Test
Repeated applications to evaluate cumulative irritation effects.

**D.5.1.3** Closed Patch Test (Occlusive or Semi-Occlusive)
Apply material using patches for defined exposure periods (e.g. 4 h, 24 h, or 48 h).

**D.5.1.4** Repeat Application Patch Test
Repeated occlusive applications to assess cumulative irritation potential.

### D.5.2 Test Procedure

**D.5.2.1** Application

i. Assess baseline skin condition before first application.
ii. Randomize test sites where appropriate.
iii. Apply measured amounts sufficient to cover the test area without overflow.
iv. Maintain consistent patch placement during repeated studies.

**D.5.2.2** Exposure Conditions

i. Define exposure duration, concentration, and application type in the protocol.
ii. Increase exposure progressively only if previous stages show acceptable tolerance.

**D.5.2.3** Removal of Test Material

Remove materials gently without rubbing to avoid cross-contamination or mechanical irritation.

## D.6 Evaluation of Skin Responses

### D.6.1 Visual Assessment

i. Assess treatment sites at predefined intervals using a standardized scoring scale.
ii. Evaluate erythema, oedema, dryness, and related skin reactions.
iii. Use the same trained assessor and consistent lighting conditions throughout the study.

### D.6.2 Instrumental Assessment

Where applicable, objective measurements may include:

i. Transepidermal Water Loss (TEWL);
ii. Redness intensity measurements;
iii. Laser Doppler flowmetry.

Measurements shall be conducted under controlled environmental conditions with calibrated instruments.

## D.7 Data Analysis and Interpretation

i. Perform statistical analyses as specified in the study protocol.
ii. Compare responses of test materials with reference or control materials.
iii. Interpret results based on irritation severity, exposure conditions, and cumulative effects.

## Bibliography

Working Group to identify and acknowledge useful literature used in the preparation of this standard.

SANS 288:2013, *Anti-bacterial liquid toilet soap for medical use*

EAS 766-2:2013, *Kenya Standard — Antibacterial toilet soap — Part 2: Liquid — Specification*