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

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Table of changes

Change No.	Date	Scope

Foreword

This South African standard was prepared by National Committee SABS/TC 217, *Cosmetics*, in accordance with procedures of the South African Bureau of Standards, in compliance with annex 3 of the WTO/TBT agreement.

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Compliance with this document cannot confer immunity from legal obligations.

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

1 Scope

This standard describes a method for testing and comparing the microbial inhibition properties of cosmetic soap bars and liquid hand and body washes.

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. Information on currently valid national and international standards can be obtained from the South African Bureau of Standards.

SANS 636, *Disinfectants based on quaternary ammonium compounds*.

SANS 7218/ISO 7218, *Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations*.

3 Principle

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

4 Test method

4.1 General

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

Only media, reagents, equipment and test organisms that comply with the requirements and guidelines given in SANS 636 and SANS 7218 shall be used.

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4.2 Accuracy

Except where otherwise specified, allow the following tolerances:

a) on temperatures ..... ± 2 °C;

b) on masses ..... ± 1,0 %;

c) on volumes ..... ± 1,0 %; and

d) on pH values ..... 0,1 pH unit.

4.3 Culture medium, reagents, reference cultures and controls

4.3.1 Culture medium

NOTE 1 The culture medium listed in 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.

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

4.3.1.2 Preparation

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

4.3.1.2.2 Make up to 1 L.

4.3.1.2.3 Adjust the pH so that after sterilization the pH value is 6,6 ± 0,05.

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.

4.3.2 Horse serum

Sterile inactivated horse serum that is free from preservatives.

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4.3.3 Reference cultures

4.3.3.1 Test organisms

Use the following test organisms:

a) *Staphylococcus aureus* ..... Sta 10 ATCC 6538;

b) *Escherichia coli* ..... Esc 20 ATCC 8739; and

c) *Pseudomonas aeruginosa* ..... Pse 16 ATCC 15442.

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

4.3.3.2 Preparation of test organism suspensions

Maintain the test organisms and prepare the cultures as described in SANS 636. Using sterile water as the diluent, prepare suspensions of the test organisms as described in SANS 636, but containing 10^5 cfu/mL ± 10^4 cfu/mL.

4.3.4 Controls

4.3.4.1 Positive control

Natural honey with no additives, e.g. colourants.

4.3.4.2 Negative control

Sterile deionised water.

4.4 Procedure

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

4.4.2 Using a 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 4.3.1.1 and allow to solidify.

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.

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

4.4.5 At the end of this period (see 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 millimetre.

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

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

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

5 Interpretation of results

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.

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.

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