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DRAFT EAST AFRICAN STANDARD

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

EAST AFRICAN COMMUNITY

Second Edition 2021

Copyright notice

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.

The Community has established an East African Standards Committee (EASC) mandated to develop and issue East African Standards (EAS) and other deliverables. The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the public and private sector organizations in the community.

East African Standards are developed through Technical Committees that are representative of key stakeholders including government, academia, consumer groups, private sector and other interested parties. 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 Principles and procedures for development of East African Standards.

East African Standards and other deliverables 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.

The committee responsible for this document is Technical Committee EASC/TC 074, *Surface active agents*

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

This second edition cancels and replaces the first edition (EAS 794:2013), which has been technically revised.

Introduction

Cosmetic soap bars and liquid hand and body washes normally have antibacterial agents incorporated into them. They not only clean the skin, but also reduce drastically the bacterial count on the skin. This prevents skin infections and perspiration odour caused by the decomposition of sweat by bacteria. They are especially effective against *Staphylococcus* and similar bacteria which have the habit of residing in the under layers of the skin.

This standard specifies a method of test for determination of the microbial inhibition of cosmetic soap bars and liquid hand and body washes.

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

1 Scope

This Draft East African Standard prescribes a method for testing and comparing the microbial inhibition properties of cosmetic soap bars and liquid hand and body washes.

2 Principle

When soap bars and liquid hand and body washes that have antimicrobial properties are inoculated into medium No.1 (see 3.2.1), 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.

3 Test method

3.1 General

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

3.2 Culture medium, reagents, reference cultures and controls

3.2.1 Culture medium

The culture medium used shall be Medium No. 1 or any other suitable agar medium can be used for the test.

3.2.1.1 Ingredients

The ingredients for Medium No. 1 shall include the following:

a) agar, 15.0 g;

b) beef extract, 15.0 g;

c) glucose, 1.0 g;

d) pancreatic digest of casein, 4.0 g;

e) peptone, 6.0 g;

f) yeast extract, 3.0 g; and

g) water, 1 000 mL.

NOTE 1 The culture medium 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

3.2.1.2 Preparation

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

3.2.1.2.2 Make up to one litre.

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

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

3.2.2 Horse serum

Sterile inactivated horse serum that is free from preservatives shall be used.

3.2.3 Reference cultures

3.2.3.1 Test organisms

Use the following test organisms or their equivalents

- *Staphylococcus aureus*: ATCC 6538;
- *Escherichia coli:* ATCC 8739; and
- *Pseudomonas aeruginosa*: ATCC 15442.

NOTE Other reference organisms may also be used in addition to the ones specified in 3.2.3.1.

3.2.3.2 Preparation of test organism suspensions

3.2.3.2.1 Nutrient medium

3.2.3.2.1.1 Ingredients

The ingredients for the nutrient medium shall include the following:

- peptone: 5.0 g;
- sodium chloride: 5.0 g;
- yeast extract: 2.0 g;
- beef extract: 1.0 g; and
- water: 1 000 mL.

NOTE The test organisms should be sub-cultured from a newly opened freeze-dried culture or recently received agar culture into bottles of 10 mL nutrient medium.

3.2.3.2.1.2 Procedure

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 ± 3 °C for 15 min. Incubate the bottles at 37 °C ± 2 °C for 24 h. Subculture onto nutrient agar slopes. Incubate the slopes at 37 °C ± 2 °C for 24 h.

3.2.3.2.2 Nutrient agar

3.2.3.2.2.1 Ingredients

The ingredients for nutrient agar shall include the following:

- 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

3.2.3.3.2.2 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. From each of these slope cultures, prepare four subcultures (stock cultures) of each test organism onto 10 mL nutrient agar slopes. Incubate the stock cultures at 37 °C ± 2 °C for 24 h and then store in a refrigerator maintained at 4 °C ± 2 °C, except for *Pseudomonas aeruginosa* which is stored at ambient temperature. 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.

3.2.3.3 Preparation of cultures for test suspensions

3.2.3.3.1 For each of the test organisms, inoculate a nutrient agar slope from a stock culture and incubate at 37 °C for 24 h.

3.2.3.3.2 For the test, use a 24 h culture that has been sub-cultured for two successive days. After six subcultures, restart the process using a fresh stock culture.

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

3.2.3.3.3 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 conical 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 105 cfu/mL ± 104 cfu/mL. Use the suspension within 3 h of preparation.

3.2.4 Control

3.2.4.1 Positive control

This shall be natural honey with no additives, for example colourants.

3.2.4.2 Negative control

This shall be sterile deionised or sterile distilled water.3.4 Procedure

3.3.1 Melt the contents of a bottle of the medium No. 1 (see 3.2.1.1) and cool to 45 ˚C ± 2 ˚C. Add 1 mL of the sterile inactivated horse serum (see 3.2.2) and 1 mL of *S. aureus* test organism suspension prepared in accordance with 3.2.3.2. Mix well and avoid the formation of air bubbles, then pour about 25 ml to 30 ml of the mixture into a Petri dish of 90 mm diameter or about 60 ml to 70 ml into a 150 - mm Petri dish and allow to solidify.**3.3.2** 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 3.2.1.1 and allow to solidify.

3.3.3 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.

3.3.4 Incubate the petri dish at 37 ˚C ± 1 ˚C for 18 h to 24 h.

3.3.5 At the end of this period (see 3.4.4), remove the Petri dish from the incubator. Measure the diameter of the zone of inhibition diagonally across the well to the nearest millimetre

3.3.6 Take the average of the five diameters and record this to the nearest millimetres**.**

3.3.7 Repeat the procedure described in 3.3.1 to 3.3.6 (inclusive) using the *E. coli* and *P. aeruginosa* test suspensions successively.

3.3.8 Repeat the procedure described in 3.3.1 to 3.3.6 (inclusive) for the positive control and the negative control.

4 Interpretation of results

4.1 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.

4.2 If the zones are not clearly defined for one of the reference organisms (for example, hazy, incomplete zone, or distorted shape), the test shall be repeated for the specified reference organism.