



EAST AFRICAN STANDARD

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

EAST AFRICAN COMMUNITY

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Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in East Africa. It is envisaged that through harmonized standardization, trade barriers which are encountered when goods and services are exchanged within the Community will be removed.

In order to meet the above objectives, the EAC Partner States have enacted an East African Standardization, Quality Assurance, Metrology and Test Act, 2006 (EAC SQMT Act, 2006) to make provisions for ensuring standardization, quality assurance, metrology and testing of products produced or originating in a third country and traded in the Community in order to facilitate industrial development and trade as well as helping to protect the health and safety of society and the environment in the Community.

East African Standards are formulated in accordance with the procedures established by the East African Standards Committee. The East African Standards Committee is established under the provisions of Article 4 of the EAC SQMT Act, 2006. The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the procedures of the Community.

Article 15(1) of the EAC SQMT Act, 2006 provides that “Within six months of the declaration of an East African Standard, the Partner States shall adopt, without deviation from the approved text of the standard, the East African Standard as a national standard and withdraw any existing national standard with similar scope and purpose”.

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

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

2 Normative reference

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EAS YYY, *Specifications for QAC based aromatic disinfectant liquid*

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.

4.2 Accuracy

Except where otherwise specified, all the following tolerances:

- on temperatures $\pm 2^{\circ}\text{C}$;
- on masses $\pm 1.0\%$;
- On volumes $\pm 1.0\%$; and
- 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

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—	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 \pm 0.2 mL volumes into suitable containers and sterilize in an autoclave for 15 min \pm 0.5 min at 121 °C.

4.3.2 Horse serum

Sterile inactivated horse serum that is free from preservatives.

4.3.3 Reference cultures

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 4.3.3.1.

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 (4.3.3.2.1)

4.3.3.2.1 Nutrient medium

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

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.

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

4.3.3.3.1 Nutrient agar**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

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.

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 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.

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.

4.3.3.6 Preparation of cultures for test suspensions

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

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 \pm 10^4 cfu/mL. Use the suspension within 3 h of preparation.

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4.3.4 Control

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 ± 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 ± 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 millimeter

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

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.

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.

