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Surface active agents — Microbiology — Microbiological test methods for liquid hand dishwashing

Agents de surface — Microbiologie — Méthodes d'essai microbiologique pour les détergents liquides de lavage de vaisselle à la main

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Foreword

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This document was prepared by Technical Committee ISO/TC 91, *Surface active agents*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Surface active agents — Microbiology — Microbiological test methods for liquid hand dishwashing

1 Scope

This document provides microbiological test methods for enumeration and detection of aerobic mesophilic bacteria, detection of *Escherichia coli* and *Pseudomonas aeruginosa* in liquid hand dishwashing.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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

ISO 21148, *Cosmetics — Microbiology — General instructions for microbiological examination*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
liquid hand dishwashing
liquid detergent which is used for dishwashing by hand

3.2
product
portion of an identified liquid hand dishwashing product received in the laboratory for testing

3.3
sample
portion of the product (at least 1 g or 1 ml) which is used in the test to prepare the initial suspension

3.4
initial suspension
suspension (or solution) of the sample in a defined volume of an appropriate enrichment broth

3.5
sample dilution
dilution of the initial suspension

3.6
aerobic mesophilic bacteria
mesophilic bacteria growing aerobically under the conditions specified in this document

3.7
Pseudomonas aeruginosa
gram-negative rod (bacilli), motile; smooth colonies pigmented (light brown or greenish)

Note 1 to entry: The main characteristics for identification are growth on selective cetrimide agar medium, oxidase positive, production of diffusible fluorescent pigments and production of a soluble phenazine pigment (pyocyanin) in suitable media.

3.8
Escherichia coli
gram-negative rod (bacilli), motile, smooth colonies

Note 1 to entry: The main characteristics for identification are catalase positive, oxidase negative, fermentation of lactose, production of indole, growth on selective medium containing bile salts with characteristic colonies.

3.9
enrichment broth
non-selective liquid medium containing suitable neutralizers and/or dispersing agents and validated for the product under test

4 Principle

This document provides enumeration and detection of aerobic mesophilic bacteria on a non-selective agar medium and enrichment medium, detection of *Escherichia coli* and *Pseudomonas aeruginosa* by presence or absence of bacterial growth after enrichment.

Microorganisms to be tested might be different from country to country according to the practices or regulations. Users can choose enumeration and/or detection methods for those microorganisms which are mentioned in this document based on their needs.

In order to ensure product quality and safety for consumers, it is advisable to perform appropriate microbiological risk analysis so as to determine the type of the product to which this document is applicable. For example, products considered to present a low microbiological risk include those with low water activity, those with extreme pH values, etc. (see ISO 29621).

Alternative microbiological methods may be substituted for the tests presented here provided that their equivalence has been demonstrated or the method has been otherwise validated.

The possible inhibition of microbial growth by the sample shall be neutralized to allow the detection of viable microorganism. In all cases the neutralization of the antimicrobial properties of the product shall be checked.

5 Diluents and culture media

5.1 General

General recommendations for microbiological examinations are specified in ISO 21148.

The following neutralizer, diluents and culture media are suitable for enumeration of aerobic mesophilic bacteria. Other diluents, neutralizers and culture media may be used if they have been demonstrated to be suitable for use.

5.2 Neutralizing diluent and diluents

5.2.1 General

The diluent is used to disperse the sample. It may contain neutralizers if the specimen to be tested has antimicrobial properties. The efficacy of the neutralization shall be demonstrated before the

determination or detection of the count. Information related to the suitable neutralizers is given in Annex A.

5.2.2 Neutralizing diluent: Fluid casein digest — soy lecithin — polysorbate 20 medium (SCDLP 20 broth)

5.2.2.1 Composition

Pancreatic digest of casein,	20,0 g
Soy lecithin,	5,0 g
Polysorbate 20,	40,0 ml
Water,	1 000 ml

5.2.3 Diluent for the bacterial suspension (Tryptone sodium chloride solution)

5.2.3.1 Composition

Tryptone, pancreatic digest of casein,	1,0 g
Sodium chloride,	8,5 g
Water,	1 000 ml

5.2.3.2 Preparation

Dissolve the components in the water by mixing while heating. Dispense into suitable containers. Sterilize in the autoclave at 121 °C for 15 min. After sterilization, the pH shall be equivalent to 7,0 ± 0,2 when measured at room temperature.

5.3 Culture medium for counting

5.3.1 General

Culture media may be prepared as follows, or from dehydrated culture media according to the instructions of the manufacturer. Ready-to-use media may be used when their composition and/or growth yields are comparable to those of the formulae given herein.

5.3.2 Soybean-casein digest agar medium (SCDA) or tryptic soy agar (TSA)

5.3.2.1 Composition

Pancreatic digest of casein,	15,0 g
Papaic digest of soybean meal,	5,0 g
Sodium chloride,	5,0 g
Agar,	15,0 g
Water,	1 000 ml

5.3.2.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by mixing while heating. Dispense the medium into suitable containers. Sterilize in the autoclave at 121 °C for 15 min. After sterilization and cooling down, the pH shall be equivalent to 7,3 ± 0,2 when measured at room temperature.

5.3.3 Other medium for counting

5.3.3.1 Eugon LT 100 agar medium

5.3.3.2 Composition

Pancreatic digest of casein,	15,0 g
Papaic digest of soybean meal,	5,0 g
L-cystine,	0,7 g
Sodium chloride,	4,0 g
Sodium sulphite,	0,2 g
Glucose,	5,5 g
Egg lecithin,	1,0 g
Polysorbate 80,	5,0 g
Octoxynol 9,	1,0 g
Agar,	15,0 g
Water,	1 000 ml

5.3.3.3 Preparation

Dissolve successively polysorbate 80, octoxynol 9 and egg lecithin into boiling water until their complete dissolution. Dissolve the other components by mixing while heating. Mix gently to avoid foam. Dispense the medium into suitable containers. Sterilize in the autoclave at 121 °C for 15 min. After sterilization and cooling down, the pH shall be equivalent to 7,0 ± 0,2 when measured at room temperature.

5.4 Culture medium for detection

5.4.1 General

An enrichment broth shall be used for bacterial detection.

The enrichment broth is used to disperse the sample and to increase the initial microbial population. It may contain neutralizers if the specimen to be tested has antimicrobial properties.