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Cosmetics — Microbiology — Detection of specified and non-specified microorganisms

Cosmétiques — Microbiologie — Détection des micro-organismes spécifiés et non spécifiés

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 217, *Cosmetics*.

This second edition cancels and replaces the first edition (ISO 18415:2007), of which it constitutes a minor revision with the following changes:

— in the Scope, "see ISO 29621" has been added and the reference has been added to the Bibliography;

— in the Scope, "used" has been changed to "substituted" and "validated" has been changed to "shown to be suitable";

— in 3.8, the term "validated" has been changed to "demonstrated to be suitable";

— in Clause 4, the term "validated" has been changed to "demonstrated";

— in 5.1, "specifications" has been changed to "instructions";

— in 5.1, the phrase "are validated" has been changed to "have been demonstrated to be suitable";

— in 5.2.1, 5.3.3.1, 11.3.1, 11.3.2, instances of the term "validation" and in the heading title of 11.3.3 have been changed to "suitability test";

— in 11.3, the term "validation" in the heading title has been changed to "suitability";

— in 11.3.3, instances of "validated" have been changed to "satisfactory";

— in Clause 12 f), the term "validation" has been changed to "demonstration of the suitability".

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Introduction

Microbiological examinations of cosmetic products are carried out according to an appropriate microbiological risk analysis in order to ensure their quality and safety for consumers.

Microbiological risk analysis depends on several parameters such as:

— potential alteration of cosmetic products;

— pathogenicity of microorganisms;

— site of application of the cosmetic product (hair, skin, eyes, mucous membranes);

— type of user (adults, children including under 3 years).

For cosmetics and other topical products, the detection of skin pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* may be relevant because they can cause skin or eye infection. The detection of other kinds of microorganisms might be of interest since these microorganisms (including indicators of faecal contamination e.g. *Escherichia coli*) suggest hygienic failure during manufacturing process.

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Cosmetics — Microbiology — Detection of specified and non-specified microorganisms

1 Scope

This document gives general guidelines for the detection and identification of specified microorganisms in cosmetic products as well as for the detection and identification of other kinds of aerobic mesophilic non-specified microorganisms in cosmetic products.

Microorganisms considered as specified in this document might differ from country to country according to national practices or regulations. Most of them considered as specified microorganisms include one or more of the following species: *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*.

In order to ensure product quality and safety for consumers, it is advisable to perform an appropriate microbiological risk analysis to determine the types of cosmetic products to which this document is applicable. Products considered to present a low microbiological risk (see ISO 29621) include those with low water activity, hydro-alcoholic products, extreme pH values, etc.

The method described in this document is based on the detection of microbial growth in a non-selective liquid medium (enrichment broth) suitable to detect microbial contamination, followed by isolation of microorganisms on non-selective agar media. Other methods can be appropriate depending on the level of detection required.

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In this document specific indications are given for identification of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. Other microorganisms that grow under the conditions described in this document may be identified by using suitable tests according to a general scheme (see Annex A). Other standards (e.g. ISO 18416, ISO 21150, ISO 22717, ISO 22718) may be appropriate.

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Because of the large variety of cosmetic products within this field of application, this method might not be suited in every detail to some products (e.g. certain water-immiscible products). Other methods (e.g. automated) can be substituted for the tests presented here provided that their equivalence has been demonstrated or the method has been otherwise shown to be suitable.

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 21148:2017, *Cosmetics — Microbiology — General instructions for microbiological examination*

EN 12353, *Chemical disinfectants and antiseptics — Preservation of test organisms used for the determination of bactericidal (including Legionella), mycobactericidal, sporicidal, fungicidal and virucidal (including bacteriophages) activity*

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:

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

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

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3.1
product
portion of an identified cosmetic product received in the laboratory for testing

3.2
sample
portion of the *product* (3.1) (at least 1 g or 1 ml) that is used in the test to prepare the *initial suspension* (3.3)

3.3
initial suspension
suspension (or solution) of the *sample* (3.2) in a defined volume of an appropriate *enrichment broth* (3.8)

3.4
sample dilution
dilution of the *initial suspension* (3.3)

3.5
aerobic mesophilic microorganism
mesophilic bacterium or yeast growing aerobically under the conditions specified in this document

Note 1 to entry: In the described conditions, other types of microorganism (e.g. moulds) are detectable.

3.6
specified microorganism
aerobic mesophilic bacterium or yeast undesirable in a cosmetic product and recognized as a skin pathogen species that may be harmful for human health or as an indication of hygienic failure in the manufacturing process

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3.6.1
Pseudomonas aeruginosa
Gram-negative rod (bacilli), motile, smooth colonies pigmented brown or greenish

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

Note 2 to entry: *Pseudomonas aeruginosa* can be isolated from a wide variety of environmental sources, especially in water and has a very high potential to spoil many different substrates. It can produce infections of human skin or eye areas. It is undesirable in cosmetic products for its potential pathogenicity and its capacity to affect the physico-chemical properties of the cosmetic formula.

3.6.2
Escherichia coli
Gram-negative rod (bacilli), motile, smooth colonies

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

Note 2 to entry: *Escherichia coli can* be isolated from the moist environmental sources (air, water, soil) and is a faecal contamination indicator.

3.6.3
Staphylococcus aureus
Gram-positive cocci, mainly aggregated in grape-like clusters, smooth colonies generally pigmented in yellow

Note 1 to entry: The main characteristics for identification are growth on a specific selective medium, catalase positive, coagulase positive.

Note 2 to entry: *Staphylococcus aureus* is an opportunistic pathogen for humans, which often can be also present on the skin of healthy individuals without causing them any apparent illness. It is a specified microorganism and undesirable in cosmetic products.

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3.6.4
Candida albicans
yeast that forms white to beige, creamy and convex colonies on the surface of a non-selective agar medium

Note 1 to entry: The main characteristics for identification are production of germ tube and/or pseudomycelium and chlamydospore when the test is performed following the method specified in this document.

3.7
non-specified microorganism
aerobic mesophilic bacterium or yeast found in cosmetic products, not defined in 3.6

3.8
enrichment broth
non-selective liquid medium containing suitable neutralizers and/or dispersing agents and demonstrated to be suitable for the *product* (3.1) under test

4 Principle

The first step of the procedure is to perform an enrichment by using a non-selective broth medium to increase the number of microorganisms without the risk of inhibition by the selective ingredients that are present in selective/differential growth media.

The following steps (isolation and identification) are performed according to need by using appropriate conditions of incubation and suitable identification test, as described in this document.

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The possible inhibition of microbial growth by the sample shall be neutralized to allow the detection of viable microorganisms[9]. In all cases and whatever the methodology, the neutralization of the antimicrobial properties of the product shall be checked and demonstrated[9][10][11].

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5 Diluents and culture media

5.1 General

General instructions are given in ISO 21148. When water is mentioned in this document, use distilled water or purified water as specified in ISO 21148.

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. The efficacy of the neutralization shall be demonstrated (see Clause 11). Information relative to suitable neutralizers is given in Annex C.

The enrichment broth (see 5.3.2.1) or any of the ones listed in Annex B is suitable for checking the presence of specified and non-specified microorganisms in accordance with this document provided that they have been demonstrated to be suitable in accordance with Clause 11.

Other diluents and culture media may be used if it has been demonstrated that they are suitable for use.

5.2 Diluent for the microbial suspension (tryptone sodium chloride solution)

5.2.1 General

The diluent is used for the preparation of bacteria and yeast suspensions used for the suitability test procedure (see Clause 11).

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5.2.2 Composition

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

5.2.3 Preparation

Dissolve the components in 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 media

5.3.1 General

Culture media may be prepared as follows, or from dehydrated culture media according to the manufacturer's instructions.

Ready-to-use media may be used when their composition and/or growth yields are comparable with those of the formulae given herein.

5.3.2 Enrichment broth

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5.3.2.1 Eugon LT100 broth

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5.3.2.1.1 General

This medium contains ingredients which neutralize inhibitory substances present in the sample: lecithin and polysorbate 80, and dispersing agent: octoxynol 9.

5.3.2.1.2 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 sulfite	0,2 g
glucose	5,5 g
egg lecithin	1,0 g
polysorbate 80	5,0 g
octoxynol 9	1,0 g
water	1 000 ml

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5.3.2.1.3 Preparation

Dissolve the components polysorbate 80, octoxynol 9 and egg lecithin, one after another in boiling water to complete dissolution. Dissolve the other components by mixing while heating. Dispense the medium 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.

5.3.2.2 Other enrichment broths

Other enrichment broths may be used as appropriate (see Annex B).

5.3.3 Non-selective agar medium

5.3.3.1 General

This medium is used for the isolation and detection of specified and non-specified microorganisms present in the initial suspension after enrichment and for the preparation of inoculum used in the suitability test procedure.

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

5.3.3.2.1 Composition

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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

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5.3.3.2.2 Preparation

Dissolve the components or the dehydrated complete medium in 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.

5.3.3.3 Other non-selective agar medium

Other non-selective, non-neutralizing agar media may be used (see Annex B).

6 Apparatus and glassware

The laboratory equipment, apparatus and glassware are described in ISO 21148.

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