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Cosmetics — Microbiology — Detection of *Pseudomonas aeruginosa*

Cosmétiques — Microbiologie — Détection de Pseudomonas aeruginosa

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

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The committee responsible for this document is ISO/TC 217, *Cosmetics*.

This second edition cancels and replaces the first edition (ISO 22717:2006), of which it constitutes a minor revision.

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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 the following:

— potential alteration of cosmetic products;

— pathogenicity of microorganisms;

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

— type of users (adults, children 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 infections. The detection of other kinds of microorganism might be of interest since these microorganisms (including indicators of faecal contamination e.g. *Escherichia coli*) suggest hygienic failure during the manufacturing process.

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Cosmetics — Microbiology — Detection of *Pseudomonas aeruginosa*

1 Scope

This International Standard gives general guidelines for the detection and identification of the specified microorganism *Pseudomonas aeruginosa* in cosmetic products. Microorganisms considered as specified in this International Standard might differ from country to country according to national practices or regulations.

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 product to which this International Standard is applicable. Products considered to present a low microbiological (see ISO 29621) risk include those with low water activity, hydro-alcoholic products, extreme pH values, etc.

The method described in this International Standard is based on the detection of *Pseudomonas aeruginosa* in a non-selective liquid medium (enrichment broth), followed by isolation on a selective agar medium. Other methods may be appropriate, depending on the level of detection required.

NOTE For the detection of *Pseudomonas aeruginosa*, subcultures can be performed on non-selective culture media followed by suitable identification steps (e.g. using identification kits).

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Because of the large variety of cosmetic products within this field of application, this method may not be appropriate in every detail for some products (e.g. certain water immiscible products). Other International Standards (ISO 18415) may be appropriate. Other methods (e.g. automated) may be substituted for the tests presented here provided that their equivalence has been demonstrated or the method has been otherwise shown to be suitable.

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2 Normative references

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

ISO 21148:2005, *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.

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

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

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

3.4
sample dilution
dilution of the initial suspension

3.5
specified microorganism
aerobic mesophilic bacterium or yeast that is undesirable in a cosmetic product and is recognized as a skin pathogen species that may be harmful for human health or as an indication of hygienic failure in the manufacturing process

3.6
Pseudomonas aeruginosa
gram-negative rod, motile; smooth colonies pigmented 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.

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

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3.7
enrichment broth
non-selective liquid medium containing suitable neutralizers and/or dispersing agents and demonstrated to be suitable for the product under test

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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 second step of the test (isolation) is performed on a selective medium followed by identification tests.

The possible inhibition of microbial growth by the sample shall be neutralized to allow the detection of viable microorganisms.[1] In all cases and whatever the methodology, the neutralization of the antimicrobial properties of the product shall be checked and demonstrated (see Clause 11).

5 Diluents and culture media

5.1 General

General instructions are given in ISO 21148. When water is mentioned in this International Standard, 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 B.

The enrichment broth (5.3.3.1), or any of the ones listed in Annex A, is suitable for checking the presence of *Pseudomonas aeruginosa* in accordance with this International Standard provided that it has been demonstrated to be suitable in accordance with Clause 11.

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

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

5.2.1 General

The diluent is used for the preparation of bacterial suspension used for the suitability test procedure (see Clause 11).

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 and cooling down, the pH shall be equivalent to 7,0 ± 0,2 when measured at room temperature.

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5.3 Culture media

5.3.1 General

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Culture media may be prepared using the descriptions provided below or from dehydrated culture media according to the instructions of the manufacturer. The instructions provided by the supplier of the media should be followed.

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

5.3.2 Agar medium for the suitability test (see Clause 11) [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.

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5.3.3 Enrichment broth

5.3.3.1 Eugon LT 100 broth

5.3.3.1.1 General

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

5.3.3.1.2 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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5.3.3.1.3 Preparation

Dissolve the components polysorbate 80, octoxynol 9 and egg lecithin successively into boiling water until their 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 and cooling down, the pH shall be equivalent to 7,0 ± 0,2 when measured at room temperature.

5.3.3.2 Other enrichment broths

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

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5.3.4 Selective agar medium for isolation of *Pseudomonas aeruginosa*

5.3.4.1 Cetrimide agar medium

5.3.4.1.1 Composition

— pancreatic digest of gelatin	20,0 g
— magnesium chloride	1,4 g
— potassium sulfate	10,0 g
— cetrimide (cetyltrimethylammonium bromide)	0,3 g
— agar	13,6 g
— glycerol	10,0 ml
— water	1 000 ml

5.3.4.1.2 Preparation

Dissolve all solid components in the water and add the glycerol. Heat, with frequent agitation, and boil for 1 min to effect dissolution.

Dispense in suitable flasks and sterilize at 121 °C for 15 min.

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After sterilization and cooling down, the pH shall be equivalent to 7,2 ± 0,2 when measured at room temperature.

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5.3.5 Selective agar medium for confirmation of *Pseudomonas aeruginosa*

5.3.5.1 Pseudomonas agar medium for detection of pyocyanin (Pseudomonas agar P)

5.3.5.1.1 Composition

— pancreatic digest of gelatin	20,0 g
— anhydrous magnesium chloride	1,4 g
— anhydrous potassium sulfate	10,0 g
— agar	15,0 g
— glycerol	10,0 ml
— water	1 000 ml

5.3.5.1.2 Preparation

Dissolve all solid components in the water, and add the glycerol. Heat, with frequent agitation, and boil for 1 min to effect dissolution.

Dispense in suitable flasks and sterilize at 121 °C for 15 min.

After sterilization and cooling down, the pH shall be equivalent to 7,2 ± 0,2 when measured at room temperature.

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