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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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

ISO 22717 was prepared by Technical Committee ISO/TC 217, *Cosmetics*.

Introduction

Microbiological examinations of cosmetic products shall be 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 micro-organisms;

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

— 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. The detection of other kinds of micro-organism might be of interest since these micro-organisms (including indicators of faecal contamination e.g. *Escherichia coli*) suggest hygienic failure during the manufacturing process.

Cosmetics — Microbiology — Detection of *Pseudomonas aeruginosa*

1 Scope

This International Standard gives general guidelines for the detection and identification of the specified micro-organism *Pseudomonas aeruginosa* in cosmetic products. Micro-organisms 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 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).

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 [10]) 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 validated.

2 Normative references

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.

ISO 21148:2005, *Cosmetics — Microbiology — General instructions for microbiological examination*

EN 12353, *Chemical disinfectants and antiseptics — Preservation of microbial strains used for the determination of bactericidal and fungicidal 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

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

3.4
sample dilution(s)
dilution(s) of the initial suspension

3.5
specified micro-organism
aerobic mesophilic bacteria 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 indication of hygienic failure in the manufacturing process

3.6
Pseudomonas aeruginosa
Gram-negative rod, motile; smooth colonies pigmented brown or greenish

NOTE 1 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 *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.

3.7
enrichment broth
non-selective liquid medium containing suitable neutralizers and/or dispersing agents and validated for the product 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 micro-organisms 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 micro-organisms [1]. In all cases and whatever the methodology, the neutralization of the antimicrobial properties of the product shall be checked and validated [2], [3], [4].

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 B.

The following enrichment broth is suitable for checking the presence of *Pseudomonas aeruginosa* in accordance with this International Standard provided that it is validated in accordance with Clause 11.

Other diluents and culture media may be used if they can be demonstrated to be 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 validation 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 whilst 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.

5.3 Culture media

5.3.1 General

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 may be used when their composition and/or growth yields are comparable to those of the formulas given herein.

5.3.2 Agar medium for validation (soybean–casein digest agar medium or tryptic soy agar)

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 whilst 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 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

5.3.3.1.3 Preparation

Dissolve the components, one after another, in boiling water – polysorbate 80, octoxynol 9 and egg lecithin until their complete dissolution. Dissolve the other components by mixing whilst 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 $7,0 \pm 0,2$ when measured at room temperature.

5.3.3.2 Other enrichment broths

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

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

— glycerin 10,0 ml

— water 1 000 ml

5.3.4.1.2 Preparation

Dissolve all solid components in the water, and add the glycerin. 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.

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

— glycerin 10,0 ml

— water 1 000 ml

5.3.5.1.2 Preparation

Dissolve all solid components in the water, and add the glycerin. 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.

6 Apparatus and glassware

The laboratory equipment, apparatus and glassware shall be as described in ISO 21148.