SLOVENSKI STANDARD
oSIST prEN ISO 21149:2017

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Kozmetika - Mikrobiologija - Ugotavljanje prisotnosti in števila aerobnih mezofilnih bakterij (ISO/FDIS 21149:2017)

Cosmetics - Microbiology - Enumeration and detection of aerobic mesophilic bacteria (ISO/FDIS 21149:2017)

Kosmetische Mittel - Mikrobiologie - Zählung und Nachweis von aeroben mesophilen Bakterien (ISO/FDIS 21149:2017)

Cosmétiques - Microbiologie - Dénombrement et détection des bactéries aérobies mésophiles (ISO/FDIS 21149:2017)

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Cosmetics — Microbiology — Enumeration and detection of aerobic mesophilic bacteria

Cosmétiques — Microbiologie — Dénombrement et détection des bactéries aérobies mésophiles

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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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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 World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

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

This second edition cancels and replaces the first edition (ISO 21149:2006), of which it constitutes a minor revision with the following changes:

— in 4.1, "validated" has been changed to "demonstrated";

— in 4.3, "validated" has been changed to "described";

— in 9.3.2.1, 9.3.2.2 and 9.3.2.3, "validated" has been changed to "described";

— in 9.3.2.3, "procedure developed during the validation" has been changed to "suitability test procedure";

— in 9.4.1, "validation" has been changed to "suitability test";

— in 12.2.1, "validated according to the chosen method" has been changed to "demonstrated to be suitable for the chosen method";

— in 13.3 and 13.4, "validation" has been changed to "suitability";

— in 13.3.2, 13.3.3 and 13.3.4, "validation" has been changed to "suitability";

— in 13.3.2, 13.3.3 and 13.3.4, "if the validation count is at least 50 % (0,3 log) of the control count" has been changed to "if the count is at least 50 % of the control";

— in 13.4.1, instances of "validation test" have been changed to "suitability test";

— in 13.4.2, instances of "validation plate" have been changed to "suitability test plate";

— in 13.5, "validation results" has been changed to "suitability test results" and "validation plates" has been changed to "suitability test plates";

— in Clause 14 f), "validation of the method" has been changed to "demonstration of the suitability";

— in A.1, B.1 and C.1, “validated” has been changed to “demonstrated to be suitable”.

Cosmetics — Microbiology — Enumeration and detection of aerobic mesophilic bacteria

1 Scope

This document gives general guidelines for enumeration and detection of mesophilic aerobic bacteria present in cosmetics

— by counting the colonies on agar medium after aerobic incubation, or

— by checking the absence of bacterial growth after enrichment.

Because of the large variety of cosmetic products within this field of application, this method may not be appropriate for some products in every detail (e.g. certain water immiscible products). 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.

If needed, microorganisms enumerated or detected may be identified using suitable identification tests described in the standards given in the Bibliography.

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

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:—[1)], *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

3.1
aerobic mesophilic bacteria
mesophilic bacteria growing aerobically under the conditions specified in this document

Note 1 to entry: In the described conditions, other types of microorganisms (e.g. yeast, mould) can be detected.

1) To be published.

3.2
product
portion of an identified cosmetic 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 a sample in a defined volume of an appropriate liquid (diluent, neutralizer, broth or combination of them)

3.5
sample dilution
dilution of the initial suspension

4 Principle

4.1 General

This method involves enumeration of colonies on a non-selective agar medium or by the presence or absence of bacterial growth after enrichment. The possible inhibition of microbial growth by the sample shall be neutralized to allow the detection of viable microorganism[7]. In all cases and whatever the methodology, the neutralization of the antimicrobial properties of the product shall be checked and demonstrated (see Clause 13)[8][9][10].

4.2 Plate count

Plate count consists of the following steps.

a) Preparation of poured plates or spread plates, using a specified culture medium, and inoculation of the plates using a defined quantity of the initial suspension or dilution of the product.

b) Aerobic incubation of the plates at 32,5 °C ± 2,5 °C for 72 h ± 6 h.

c) Counting the number of colony forming units (CFU) and calculation of the number of aerobic mesophilic bacteria per millilitre or per gram of product.

4.3 Membrane filtration

Membrane filtration consists of the following steps.

a) Transfer a suitable amount of the sample prepared as described in Clause 13 in the filtration apparatus wetted with a small volume of an appropriate sterile diluent, filter immediately and wash according to the validated procedure (see 13.3.4). Transfer the membrane filter onto the surface of the specified agar medium as specified in ISO 21148.

b) Aerobic incubation of the membranes at 32,5 °C ± 2,5 °C for 72 h ± 6 h.

c) Counting the number of colony forming units (CFU) and calculation of the number of aerobic mesophilic bacteria per millilitre or per gram of product.

4.4 Detection of bacteria by enrichment

Detection of bacteria by enrichment consists of the following steps.

a) Incubation at 32,5 °C ± 2,5 °C for at least 20 h of a defined quantity of the initial suspension in a non-selective liquid medium containing suitable neutralizers and/or dispersing agents.

b) Transfer of a defined quantity of the previous suspension on non-selective solid agar medium.

c) Aerobic incubation at 32,5 °C ± 2,5 °C for 48 h to 72 h.

d) Detection of growth and expression of results as “presence/absence” of aerobic mesophilic bacteria per sample *S* of product.

5 Diluents, neutralizers and culture media

5.1 General

General specifications are given in ISO 21148. When water is used in a formula, use distilled water or purified water as specified in ISO 21148.

The following diluents, neutralizers and culture media are suitable for enumeration and detection 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 diluents 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 of the count (see Clause 13). Information relative to suitable neutralizers is given in Annex D.

5.2.2 Neutralizing diluents

5.2.2.1 Fluid casein digest–soy lecithin–polysorbate 20 medium (SCDLP 20 broth)

5.2.2.1.1 Composition

Pancreatic digest of casein	20,0 g
Soy lecithin	5,0 g
Polysorbate 20	40,0 ml
Water	960,0 ml

5.2.2.1.2 Preparation

Dissolve the polysorbate 20 in 960 ml of water by mixing while heating in a water bath at 49 °C ± 2 °C. Add pancreatic digest of casein and soy lecithin. Heat for about 30 min to obtain solution. Mix and dispense the medium into suitable containers. Sterilize in the autoclave at 121 °C for 15 min. After sterilization, the pH shall be equivalent to 7,3 ± 0,2 when measured at room temperature.

5.2.2.2 Other neutralizing diluents

Other neutralizing diluents may be used as appropriate (see Annex A and Annex D).

5.2.3 Diluent

5.2.3.1 Fluid A

5.2.3.1.1 Composition

Peptic digest of animal tissue	1,0 g
Water	1 000 ml

5.2.3.1.2 Preparation

Dissolve 1 g of peptone in water to make 1 l. Heat with frequent agitation. Dispense into suitable containers. Sterilize in the autoclave at 121 °C for 15 min. After sterilization, the pH shall be equivalent to 7,1 ± 0,2 when measured at room temperature.

5.2.3.2 Other diluents

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

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

5.3.1 Composition

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

5.3.2 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.

5.4 Culture media

5.4.1 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 formulas given herein.

