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Cosmetics — Microbiology — Evaluation of the antimicrobial protection of a cosmetic product

Cosmétiques — Microbiologie — Évaluation de la protection antimicrobienne d'un produit cosmétique

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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 11930 was prepared by Technical Committee ISO/TC 217, *Cosmetics*.

This corrected version of ISO 11930:2012 incorporates the following correction:

— in Table B.1, in the Criteria A row, final column (T28), the words "and NI" have been added.

Introduction

This International Standard is to be used in the overall evaluation of the antimicrobial protection of a cosmetic product.

The antimicrobial protection of a product can come from many sources:

— chemical preservation;

— inherent characteristics of the formulation;

— package design;

— manufacturing process.

This International Standard defines a series of steps to be taken when assessing the overall antimicrobial protection of a cosmetic product. A reference method for a preservation efficacy test (challenge test) along with evaluation criteria is also described in this International Standard.

The data generated by the risk assessment (see ISO 29621) or by the preservation efficacy test, or both, are to be used to establish the level of antimicrobial protection required to minimize user risk.

Cosmetics — Microbiology — Evaluation of the antimicrobial protection of a cosmetic product

1 Scope

1.1 General

This International Standard comprises:

— a preservation efficacy test;

— a procedure for evaluating the overall antimicrobial protection of a cosmetic product which is not considered low risk, based on a risk assessment described in ISO 29621.

This International Standard provides a procedure for the interpretation of data generated by the preservation efficacy test or by the microbiological risk assessment, or both.

1.2 Preservation efficacy test

This test is a reference method that is to be used to evaluate the preservation of a cosmetic formulation. It applies to cosmetic products in the market place.

This test is not required for those cosmetic products for which the microbiological risk has been determined to be low (see Annex A and ISO 29621).

This test is primarily designed for water-soluble or water-miscible cosmetic products and can require adaptation, for example to test products in which water is the internal phase. The test described in this International Standard involves, for each test micro-organism, placing the formulation in contact with a calibrated inoculum, and then measuring the changes in the micro-organism count at set time intervals for a set period and at a set temperature.

NOTE This test can be used as a guideline to develop an in-house method during the development cycle of cosmetic products. In this case, the test can be modified or extended, or both, for example to make allowance for prior data and different variables (microbial strains, media, incubation conditions exposure time, etc.). Compliance criteria can be adapted to specific objectives. During the development stage of cosmetic products, other methods, where relevant, can be used to determine the preservation efficacy of formulations.

1.3 Procedure for evaluating the antimicrobial protection of the cosmetic product

This procedure is based on careful consideration of the following points.

— Results of the preservation efficacy test. Not all cosmetic products will require a preservation efficacy test (see Annex A and ISO 29621).

— Formulation characteristics and data provided by the microbiological risk assessment (see ISO 29621). The analysis of the microbiological risk assessment is based on an overall approach. In particular, it integrates variables such as characteristics and composition of the formulation, its production conditions, the characteristics of the packaging in which the formulation will be delivered to the market place, recommendations for use of the cosmetic product and, when relevant, the area of application and the targeted user population (see Annex D).

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 16212, *Cosmetics — Microbiology — Enumeration of yeast and mould*

ISO 18415, *Cosmetics — Microbiology — Detection of specified and non-specified microorganisms*

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

ISO 21149, *Cosmetics — Microbiology — Enumeration and detection of aerobic mesophilic bacteria*

ISO 22716, *Cosmetics — Good Manufacturing Practices (GMP) — Guidelines on Good Manufacturing Practices*

ISO 29621, *Cosmetics — Microbiology — Guidelines for the risk assessment and identification of microbiologically low-risk products*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 21148 and the following apply.

3.1
cosmetic formulation
preparation of raw materials with a qualitatively and quantitatively defined composition

3.2
cosmetic product
finished cosmetic product that has undergone all stages of production, including packaging in its final container for shipment

3.3
antimicrobial protection of a cosmetic product
ability of a cosmetic product to overcome microbial contamination that might present a potential risk to the user

NOTE The overall antimicrobial protection includes preservation of the formulation, the specific manufacturing process and protective packaging.

3.4
preservation of a cosmetic formulation
set of means used to avoid microbial proliferation in a cosmetic formulation

EXAMPLES Preservatives, multifunctional compounds, hostile raw materials, extreme pH, low water-activity values.

3.5
reference method
method applied by interested parties to assess a product on the market and in case of dispute

3.6
development method
in-house method
method used during the development stage of a product before the product is put on the market

3.7
consumer
end user of a cosmetic product

4 Principle

The evaluation of the antimicrobial protection of a cosmetic product combines the following elements (see Annex A).

a) The characteristics of its formulation (see ISO 29621) or the results of the preservation efficacy test (if performed), or both.

   The preservation efficacy test is described in 5.1.

b) The characteristics of the cosmetic product in conjunction with the production conditions (see ISO 22716 and ISO 29621), the packaging materials and, if justified, recommendations for use of the product (see ISO 29621).

This International Standard describes a procedure for the interpretation of data generated by the preservation efficacy test (if appropriate) and by the microbiological risk assessment.

5 Preservation efficacy test

5.1 General

The evaluation of the preservation of a cosmetic formulation is based on inoculation of the formulation with calibrated inocula (prepared from relevant strains of micro-organisms). The number of surviving micro-organisms is measured at defined intervals during a period of 28 days. For each time and each strain, the log reduction value is calculated and compared to the minimum values required for evaluation criteria A or B (see Annex B).

When used as a reference method, procedures shall be strictly followed in order to avoid variability in results. To determine the preservation efficacy of a formulation during product development, other suitable development methods may be used (see 1.2).

Prior to the test, the microbiological quality of the product shall be determined in accordance with ISO 21149 and ISO 16212, or with ISO 18415.

NOTE The micro-organisms present in the test sample should not interfere with the recovery of the test organisms.

In the test, the neutralization of the possible antimicrobial activity of the tested sample shall be checked and demonstrated (see 5.5).

5.2 Materials, apparatus, reagents and culture media

General specifications and instructions are given in ISO 21148. When water is used in a formula, use distilled water or purified water as specified in ISO 21148:2005, 8.2.

5.2.1 Materials

Use usual microbiology laboratory equipment (see ISO 21148) and:

5.2.1.1 **Glass beads**, 3 mm to 4 mm in diameter.

5.2.1.2 **Sintered glass filter**, of porosity 2 (40 µm to 100 µm).

5.2.1.3 Roux flasks.

5.2.1.4 Sterile glass containers with closures, of suitable volumes.

5.2.1.5 Centrifuge, capable of a centrifugal force of 2 000 g.

5.2.2 Diluents, neutralizers and culture media

5.2.2.1 General

Unless otherwise specified, all reagents shall be equilibrated at ambient temperature before use. When available, ready-to-use reagents and media may be used.

5.2.2.2 Diluent

5.2.2.2.1 Composition

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

5.2.2.2.2 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.

5.2.2.2.3 Polysorbate solution (for preparation of *A. brasiliensis* spore suspension)

Prepare a solution of polysorbate 80 (0,5 g/l). Dissolve by mixing while heating until complete dissolution is achieved. Dispense the solution into suitable containers. Sterilize in the autoclave at 121 °C for 15 min.

5.2.2.3 Neutralizer

5.2.2.3.1 General

The suitability and effectiveness of the neutralizing agent with respect to the test strains used and to the tested formulation shall be demonstrated as specified in 5.5.

The neutralizer described in 5.2.2.3.2 is frequently used. Examples of other suitable neutralizers are given in Annex C.

5.2.2.3.2 Eugon LT 100 liquid broth

5.2.2.3.2.1 General

This medium contains ingredients which neutralize inhibitory substances present in the sample (lecithin and polysorbate 80) and dispersing agent octoxynol 9 (Triton X100®[1]). It may be prepared as described in 5.2.2.3.2.2, or from dehydrated culture medium, according to the manufacturer's instructions. A ready-to-use medium may also be used.

[1] Triton X100® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

5.2.2.3.2.2 Composition

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

5.2.2.3.2.3 Preparation

Dissolve successively into boiling water polysorbate 80, octoxynol 9 and egg lecithin until they are completely dissolved. Dissolve the other components by mixing while heating. Dispense the medium into suitable containers. Sterilize in the autoclave at 121 °C for 15 min. Mix well after sterilization while the liquid is still hot to redissolve settled substances. After sterilization, the pH shall be equivalent to 7,0 ± 0,2 when measured at room temperature.

5.2.2.4 Culture media

5.2.2.4.1 General

Culture media may be prepared as in 5.2.2.4.2, 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 to those of the formulae given in 5.2.2.4.2.1.

5.2.2.4.2 Culture medium for bacteria: tryptic soy agar (TSA) or soybean casein digest agar medium

5.2.2.4.2.1 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

5.2.2.4.2.2 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. Mix well after sterilization while the liquid is still hot to redissolve settled substances. After sterilization and cooling down, the pH shall be equivalent to 7,3 ± 0,2 when measured at room temperature.

5.2.2.4.3 Culture medium for *C. albicans:* Sabouraud dextrose agar medium (SDA)

5.2.2.4.3.1 Composition

Dextrose	40,0 g
Peptic digest of animal tissue	5,0 g
Pancreatic digest of casein	5,0 g
Agar	15,0 g
Water	1 000 ml

5.2.2.4.3.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by mixing while heating. Dispense the medium into suitable containers. Sterilize in an autoclave at 121 °C for 15 min. After sterilization, the pH shall be equivalent to 5,6 ± 0,2 when measured at room temperature.

5.2.2.4.4 Culture medium for *A. brasiliensis:* potato dextrose agar (PDA)

5.2.2.4.4.1 Composition

Potato infusion (see 5.2.2.4.4.2, Note 1)	200,0 g
Dextrose	20,0 g
Agar (see 5.2.2.4.4.2, Note 2)	20,0 g
Water	1 000 ml

5.2.2.4.4.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by heating. Dispense the medium into suitable containers. Sterilize in an autoclave at 121 °C for 15 min. After sterilization, the pH shall be equivalent to 5,6 ± 0,2 when measured at room temperature.

NOTE 1 To prepare potato infusion, use a commercial dehydrated form or boil 200 g sliced, unpeeled potatoes in 1 l of water for 30 min. Filter through a cheesecloth, saving the effluent.

NOTE 2 Commercially available dehydrated medium powders which contain less than 20 g/l of agar can be supplemented with extra agar to the final concentration of 20 g/l if necessary.

5.3 Microbial strains

The test shall be run using the following strains as test micro-organisms:

— *Pseudomonas aeruginosa* ATCC®9027™[2] (equivalent strain: CIP®82.118™[3] or NCIMB®8626™[4] or NBRC®13275™[5] or KCTC®2513™[6] or other equivalent national collection strain);

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2 ATCC®: American Type Culture Collection

3 CIP®: Collection de l'Institut Pasteur

4 NCIMB®: National Collection of Industrial Marine Bacteria

5 NBRC®: NITE Biological Resource Center, JP

6 KCTC®: Korean Collection for Type Cultures