INTERNATIONAL STANDARD

ISO 21322

First edition
2020-06

Cosmetics — Microbiology — Testing of impregnated or coated wipes and masks

Cosmétiques — Microbiologie — Essais sur lingettes et masques imprégnés ou enduits

Reference number
ISO 21322:2020(E)

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Published in Switzerland

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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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For an explanation of the voluntary nature of standards, 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 www.iso.org/iso/foreword.html.

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

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

For technical reasons, current standards in cosmetics microbiology may not be applicable to impregnated or coated cosmetic products, such as wipes and masks, in which there is no direct access to the formulation.

Based on their product form or delivery there is a need to adapt these standards to assess the microbiological quality of these products.

Cosmetics — Microbiology — Testing of impregnated or coated wipes and masks

1 Scope

This document gives guidance for the enumeration and/or detection of microorganisms present in a cosmetic product that is impregnated or coated onto a substrate (i.e. wipes and masks) where sampling and microbiological influence of the manufactured product presents particular challenges in terms of microbiological sampling and testing.

The principle of this document can also be applied to test similar products (e.g. cushion, impregnated sponge, etc.) or applicators (e.g. brush, puff, sponge, etc.) with modification of the procedure as appropriate.

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 11930, *Cosmetics — Microbiology — Evaluation of the antimicrobial protection of a cosmetic product*

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

ISO 18416, *Cosmetics — Microbiology — Detection of Candida albicans*

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

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

ISO 21150, *Cosmetics — Microbiology — Detection of Escherichia coli*

ISO 22717, *Cosmetics — Microbiology — Detection of Pseudomonas aeruginosa*

ISO 22718, *Cosmetics — Microbiology — Detection of Staphylococcus aureus*

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:

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

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

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

3.2
cosmetic product
cosmetic formulation (3.1) that has undergone all stages of production, including packaging in its final container, for shipment

3.3
impregnated product
product absorbed on the support

3.4
coated product
product adsorbed on the support

3.5
test sample
representative unit of the entire *cosmetic product* (3.2) for testing

4 Principle

4.1 General information

The method determines the population of viable microorganisms by enumeration of colonies on a non-selective agar medium and by the presence or absence of specified microorganisms growth after enrichment.

The method involves the following steps:

— selection of the test sample;

— selection of an appropriate method;

— recovery of microorganisms;

— enumeration of the population of viable microorganisms by filtration or plate count method;

— tests for specified microorganisms by enrichment method.

The experimental conditions shall be evaluated to ensure that the method should not affect the viability of microorganisms and the recovery of bioburden from the sample and should include the verification of the efficacy of the neutralization (see Clause 11).

In order to ensure product quality and safety for the consumer, an appropriate microbiological risk assessment should be performed to determine the types of cosmetic products to which this document is applicable (see ISO 29621:2017, Table 2).

Other methods may be substituted provided that their equivalence has been demonstrated.

4.2 Selection of the test sample

— Whenever practical, the entire unit should be used for testing with a minimum weight of 1 g. If for technical reasons the entire unit cannot be tested, a defined Unit Item Portion (UIP) is used for testing. A “UIP” is a microbiologically-representative subunit of the test sample and is referenced throughout the document.

— If the unit is < 1 g per unit then the appropriate number of units should be sampled to achieve the appropriate weight or volume.

— The weight of the test sample shall be recorded even if the results are expressed by unit.

Selection of the test sample shall be according to A.1.

4.3 Selection of the method

The method should be conducted according to an appropriate procedure based on the specifics of the product (size, volume, single unit/multi–unit package, level of bioburden. etc.) and should ensure that a representative sample is evaluated.

Selection of the method shall be according to A.1 and A.2.

4.4 Recovery of microorganisms from the test sample

The degree to which microorganisms adhere to the test sample surface is dependent on the wipe or mask in which the liquid portion of the formulation has been either impregnated or coated. Preliminary treatments may be necessary to separate microorganisms from the test sample.

Regardless of the treatment, the verification of recovery method should be performed in order to demonstrate that the method can release microorganisms from the test sample without having an adverse effect on their viability (see Clause 11).

4.5 Enumeration of aerobic mesophilic microorganisms

4.5.1 General

The enumeration of aerobic mesophilic microorganisms includes bacteria, yeasts and moulds.

Based on the nature of the test sample, the volume of diluent used to immerse the test sample and the expected level of bioburden, two types of counting methods may be used:

— plate count method;

— membrane filtration method.

4.5.2 Plate count method overview

Plate count method consists of either using a pour plate or spread plate method.

Each method consists of the following steps.

— Prepare the agar plates and diluent using a non-selective agar medium for plating the diluent in which the sample was immersed.

— Incubate the plates for enumeration and/or detection.

— Count the number of colonies forming units (CFU) based on the number of aerobic mesophilic microorganisms recovered per unit or g.

4.5.3 Membrane filtration method overview

Membrane filtration consists of the following steps.

— Transfer the diluent or a defined quantity of diluent in which the test sample was immersed to a filtration apparatus wetted with a small volume of an appropriate sterile diluent.

— After filtration and rinsing, transfer the membrane filter onto the surface of plates with non-selective agar medium.

— Aerobic incubation of the plates.

— Count the number of colony forming units (CFU) and calculate of the number of aerobic mesophilic microorganisms per g or unit.

4.6 Detection of specified microorganisms by enrichment method

The objective of the enrichment method is to incubate a test sample in a non-selective broth medium to increase the number of microorganisms that are present in a test sample.

— The first step of an enrichment method is to incubate the test sample in a non-selective broth medium to increase the number of microorganisms present in the test sample.

— The second step of an enrichment method is to isolate specified microorganisms that may be present on a test sample through the use of selective agar media followed by confirmatory identification tests for characteristic colonies. See ISO 18416, ISO 21150, ISO 22717 and ISO 22718.

5 Diluents, neutralizers and culture media

5.1 General

The diluents, neutralizers and culture media suitable for enumeration and detection of aerobic mesophilic microorganisms are described in ISO 11930, ISO 16212 and ISO 21149. Other diluents, neutralizers and culture media may be used if they have been demonstrated to be suitable for use.

Use the general instructions given in ISO 21148. When water is mentioned in this document, use distilled water or purified water as specified in ISO 21148.

5.2 Diluents and neutralizers

The diluent is used to disperse the sample. It is required that it contain neutralizers if the sample to be tested has antimicrobial properties or contains a preservative. The efficacy of the neutralization shall be demonstrated before the determination of the count (see Clause 11). Diluents and neutralizers shall be in accordance with ISO 11930, ISO 16212, ISO 18416, ISO 21149, ISO 21150, ISO 22717 and ISO 22718.

5.3 Culture media

5.3.1 Media for enumeration and detection

Culture media for enumeration and/or detection shall be in accordance with ISO 11930, ISO 16212, ISO 18416, ISO 21149, ISO 21150, ISO 22717 and ISO 22718.

5.3.2 Media for preparation of spores of *Bacillus subtilis*

See C.1.3.1.

6 Apparatus and glassware

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

7 Strains of microorganisms

The culture should be reconstituted according to the procedures provided by the supplier of the reference strain. The strains may be stored in the laboratory conforming to EN 12353 or according to another suitable method.

For testing the recovery of microorganisms on the test samples, spores of *Bacillus subtilis* ATCC 6633 (equivalent strain CIP 52.62 or NCIMB 8054 or NBRC 3134 or other equivalent national collection strain) are used.

For testing the efficacy of neutralizers, two strains representative of both Gram negative and Gram positive bacteria and a yeast are used:

— *Staphylococcus aureus* ATCC 6538 (equivalent strain: CIP 4.83 or NCIMB 9518 or NBRC 13276 or KCTC 1916 or other equivalent national collection strain);

— *Pseudomonas aeruginosa* ATCC 9027 (equivalent strain: CIP 82.118 or NCIMB 8626 or NBRC 13275 or KCTC 2513 or other equivalent national collection strain).

An alternative Gram negative strain may be *Escherichia coli* ATCC 8739 (equivalent strain: CIP 53.126 or NCIMB 8545 or NBRC 3972 or KCTC 2571 or other equivalent national collection strain).

— *Candida albicans* ATCC 10231 (equivalent strain: IP 48.72 or NCPF 3179 or NBRC 1594 or KCTC 17205, or other equivalent national collection strain).

The strains may be kept in the laboratory according to the EN 12353.

8 Handling of cosmetic products and laboratory samples

If storage is necessary, keep the products to be tested at room temperature. Do not incubate, refrigerate or freeze products and samples before or after analysis. Sampling and test procedures should follow the guidelines specified in ISO 21148 and in accordance with the procedure outlined in Clause 9.

9 Procedure

9.1 General recommendation

Use sterile equipment and aseptic technique whenever preparing the test sample and diluent.

For the preparation of an initial suspension, the time which elapses between the end of the preparation of the test sample and the moment the diluent of the initial suspension comes into contact with the culture medium shall not exceed (30 ± 15) min, unless specifically mentioned in the established protocols or documents.

The method should follow the procedure developed during the suitability test, to ensure neutralization of potential inhibitory effects (see Clause 11) and to guarantee the recovery of microorganisms.

9.2 Selection and preparation of the test sample

9.2.1 Selection of the test sample

The test sample shall weigh at least 1 g.

The test sample can be either the entire unit, or multiple units if the weight of one unit is less than 1 g, or the UIP (see A.1).

Record the exact weight of the test sample, *S,* and, the number of units, *n*.

If a UIP is used for testing, record the UIP value of the test sample (see 4.2).

9.2.2 Preparation of the initial suspension

Place the test sample (see 9.2.1) into an appropriate container, with a known volume of diluent, defined in the suitability test (see Clause 11). The test sample should be completely immersed in the diluent.

Record the value for “V”, the volume of diluent used.