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Cosmetics — Microbiology — Enumeration of yeast and mould

Cosmétiques — Microbiologie — Dénombrement des levures et des moisissures

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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 on 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 the following URL: www.iso.org/iso/foreword.html.

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

This second edition cancels and replaces the first edition (ISO 16212:2008), of which it constitutes a minor revision. The changes compared to the previous edition are as follows:

— in the Scope, "see ISO 29621" has been added and the reference has been added to the Bibliography;

— in the Scope, "used" has been changed to "substituted" and "validated" has been changed to "shown to be suitable";

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

— in 4.3, "by a valid method" has been changed to "as described in Clause 12" and "validated procedure" has been replaced by "described procedure";

— in 5.1, "specifications" has been changed to "instructions";

— in 5.2.3.1.2, "peptone" has been changed to "peptic digest of animal tissue";

— in Clause 7, "validation" has been changed to "suitability";

— in 9.3.2.1, "validated" has been changed to "demonstrated to be suitable";

— in 9.3.2.3, "prepared as validated" has been changed to "demonstrated to be suitable";

— in 11.2.1, "validated according to" has been changed to "demonstrated to be suitable for";

— in 12.3, "validation" has been changed to "suitability";

— in 12.3.2, instances of "validation" have been changed to "suitability test" and "validated" has been changed to "satisfactory";

— in 12.3.3, the first instance of "validation" has been changed to "suitability" and the second instance has been changed to "suitability test"; "validated" has been changed to "satisfactory";

— in 12.3.4, the first instance of “validation” has been changed to “suitability” and the second instance has been changed to “suitability test”; “validated” has been changed to “satisfactory”;

— in Clause 13 f), “validation” has been changed to “suitability”;

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

Cosmetics — Microbiology — Enumeration of yeast and mould

1 Scope

This document gives general guidelines for enumeration of yeast and mould present in cosmetics by counting the colonies on selective agar medium after aerobic incubation.

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

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

Yeast enumerated can be identified using suitable identification tests, for example, tests described in the standards listed in the Bibliography. Mould enumerated can be identified by other appropriate methods, if necessary.

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, *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:

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

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

3.1
yeast
single-cell fungus, which multiplies mainly vegetatively by budding, able to grow under the test conditions specified in this document

3.2
mould
mycelium forming microfungus, including spores and conidia, able to grow under the test conditions specified in this document

3.3
product
portion of an identified cosmetic product received in the laboratory for testing

3.4
sample
portion of the *product* (3.3) (at least 1 g or 1 ml) that is used in the test to prepare the initial suspension

3.5
initial suspension
suspension (or solution) of the *sample* (3.4) in a defined volume of an appropriate enrichment broth

3.6
sample dilution
dilution of the *initial suspension* (3.5)

4 Principles

4.1 General

This method involves enumeration of colonies on a selective agar medium. The possible inhibition of fungal growth by the sample shall be neutralized to allow the detection of viable microorganisms[5]. In all cases and whatever the methodology, the neutralization of the antifungicidal properties of the product shall be checked and demonstrated[6][8][9].

4.2 Plate count

Plate count consists of the following steps.

— 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.

— Aerobic incubation of the plates at 25 °C ± 2,5 °C for 3 d to 5 d.

— Counting of the number of colony-forming units (CFU) and calculation of the amount of yeast and mould per millilitre or per gram of product.

NOTE An alternative condition for incubation is 22,5 °C ± 2,5 °C for 5 d to 7 d using the culture medium without antibiotic.

4.3 Membrane filtration

Membrane filtration consists of the following steps.

— Transfer a suitable amount of the sample, prepared as described in Clause 12, in the filtration apparatus, wetted with a small volume of an appropriate sterile diluent. Filter immediately and wash according to the described procedure (see 12.3.4). Transfer the membrane filter onto the surface of the specified agar medium as specified in ISO 21148.

— Aerobic incubation of the membranes at 25 °C ± 2,5 °C for 3 d to 5 d.

— Counting of the number of colony-forming units (CFU) and calculation of the amount of yeast and mould per millilitre or per gram of product.

NOTE An alternative condition for incubation is 22,5 °C ± 2,5 °C for 5 d to 7 d using the culture medium without antibiotic.

5 Diluents, neutralizers 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 following diluents, neutralizers and culture media are suitable for enumeration of yeasts and moulds. 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 sample to be tested has antifungicidal properties. The efficacy of the neutralization shall be demonstrated before the determination of the count (see Clause 12). Information relative to suitable neutralizers is given in Annex D.

5.2.2 Neutralizing diluent

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 ml
Water	960 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 effect 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 peptic digest of animal tissue 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 yeast suspension (tryptone sodium chloride solution)

5.3.1 Composition

Tryptone, pancreatic digest of casein	1,00 g
Sodium chloride	8,50 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 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 herein.

5.4.2 Sabouraud dextrose chloramphenicol agar medium (SDCA)

5.4.2.1 Composition

Dextrose	40,0 g
Peptic digest of animal tissue	5,0 g
Pancreatic digest of casein	5,0 g
Chloramphenicol	0,050 g
Agar	15,0 g
Water	1 000 ml

5.4.2.2 Preparation

Dissolve the components (including the chloramphenicol) 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.

NOTE For known and non-contaminated products (with bacteria), the media are used without chloramphenicol.

5.4.3 Other media

Other media may be used as appropriate (see Annex C).

5.4.4 Agar medium for cultivation of reference strain: Sabouraud dextrose agar medium (SDA)

5.4.4.1 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

5.4.4.2 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.

6 Apparatus and glassware

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

7 Strain of microorganisms

For testing the efficacy of neutralizers, one yeast reference strain is used:

— *Candida albicans* ATCC[1] 10231 or equivalent strain (IP[2] 48.72 or NCPF[3] 3179 or NBRC[4] 1594 or KCTC[5] 17205 or TISTR[6] 5779) or other equivalent national collection strain.

The selected yeast strain being considered more susceptible to antifungicidal activity than moulds may be accepted as representative of fungi (yeast and mould) for the suitability of the methodology. However, in case of specific needs, the test for the efficacy of neutralizers may be performed with an additional mould reference strain, using a suitable protocol for the preparation of a calibrated inoculum (e.g. see EN 13624:2013, 5.4.1.4[3]).

The culture should be reconstituted according to the procedures provided by the supplier of the reference strain.

1) ATCC = American Type Culture Collection.

2) IP = Institut Pasteur.

3) NCPF = National Collection of Pathogenic Fungi.

4) NBRC = Biological Resource Center, NITE.

5) KCTC = Korean Collection for Type Cultures.

6) TISTR = Thailand Institute of Scientific and Technological Research.