SLOVENSKI STANDARD
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Kozmetika - Mikrobiologija - Ugotavljanje števila kvasovk in plesni (ISO/FDIS 16212:2017)

Cosmetics - Microbiology - Enumeration of yeast and mould (ISO/FDIS 16212:2017)

Kosmetische Mittel - Mikrobiologie - Zählung von Hefen und Schimmelpilzen (ISO/FDIS 16212:2017)

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

This second edition cancels and replaces the first edition (ISO 16212:2008), of which it constitutes a minor revision with the following changes:

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

— 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" has been changed to "demonstrated to be suitable";

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

— in 12.3.2, instances of "validation" have been changed to "suitability test";

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

— in 12.3.4, "validation" has been changed to "suitability";

— in 12.3.4, "validated" has been changed to "satisfactory";

— in 12.3.4, "validation" has been changed to "suitability test";

— in Clause 13 f), "validation" has been changed to "suitability".

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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 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, products with extreme pH values, 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 used for the test presented here provided that their equivalence has been demonstrated or the method has been otherwise validated.

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.

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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.

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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:

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

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

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

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

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

3.5
initial suspension
suspension (or solution) of a sample in a defined volume of an appropriate liquid (diluent, neutralizer, broth or combination thereof)

3.6
sample dilution(s)
dilution(s) of the initial suspension

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 microorganism (see Reference [5]). In all cases and whatever the methodology, the neutralization of the antifungicidal properties of the product shall be checked and satisfactory (see References [6], [8] and [9]).

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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 25 °C ± 2,5 °C for 3 d to 5 d.

c) 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.

a) Transfer of a suitable amount of the sample, prepared by a valid method, in the filtration apparatus, wetted with a small volume of an appropriate sterile diluent. Immediate filtration and washing according to the validated procedure. Transfer of the membrane filter onto the surface of the specified agar medium as specified in ISO 21148.

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

c) 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.

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5 Diluents, neutralizers and culture media

5.1 General

General specifications are given in ISO 21148. When water is mentioned 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 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)

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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

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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

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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 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.

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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

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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

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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.

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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 validation 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 13624:2003, 5.4.1.4[3]).

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

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