

Cosmetics — Microbiology — Enumeration of yeast and mould

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National foreword

This British Standard is the UK implementation of EN ISO 16212:2011. It is identical with ISO 16212:2008. It supersedes BS ISO 16212:2008, which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee CW/217, Cosmetics.

A list of organizations represented on this committee can be obtained on request to its secretary.

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English Version

**Cosmetics - Microbiology - Enumeration of yeast and mould
(ISO 16212:2008)**

Cosmétiques - Microbiologie - Dénombrement des levures
et des moisissures (ISO 16212:2008)

Kosmetik - Mikrobiologie - Zählung von Hefen und
Schimmelpilzen (ISO 16212:2008)

This European Standard was approved by CEN on 12 May 2011.

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Foreword

The text of ISO 16212:2008 has been prepared by Technical Committee ISO/TC 217 “Cosmetics” of the International Organization for Standardization (ISO) and has been taken over as EN ISO 16212:2011 by Technical Committee CEN/TC 392 “Cosmetics” the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by December 2011, and conflicting national standards shall be withdrawn at the latest by December 2011.

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Endorsement notice

The text of ISO 16212:2008 has been approved by CEN as a EN ISO 16212:2011 without any modification.

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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.

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ISO 16212 was prepared by Technical Committee ISO/TC 217, *Comestics*.

Cosmetics — Microbiology — Enumeration of yeast and mould

1 Scope

This International Standard 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 International Standard 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.

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

EN 12353, *Chemical disinfectants and antiseptics — Preservation of test organisms used for the determination of bactericidal, mycobactericidal, sporicidal and fungicidal activity*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

yeast

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

3.2

mould

mycelium forming microfungus, including spores and conidia, able to grow under the test conditions specified in this International Standard

- 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 [2]). In all cases and whatever the methodology, the neutralization of the antifungal properties of the product shall be checked and validated (see References [3], [5], [6]).

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 $25\text{ °C} \pm 2,5\text{ °C}$ 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 $22,5\text{ °C} \pm 2,5\text{ °C}$ 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 on to the surface of the specified agar medium as specified in ISO 21148.
- b) Aerobic incubation of the membranes at $25\text{ °C} \pm 2,5\text{ °C}$ 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 $22,5\text{ °C} \pm 2,5\text{ °C}$ for 5 d to 7 d using the culture medium without antibiotic.

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)

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 $49\text{ °C} \pm 2\text{ °C}$. 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 $7,3 \pm 0,2$ 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