

Cosmetics — Microbiology — Enumeration and detection of aerobic mesophilic bacteria

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National foreword

This British Standard is the UK implementation of EN ISO 21149:2009. It is identical to ISO 21149:2006. It supersedes BS ISO 21149:2006 which is withdrawn.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 and detection of
aerobic mesophilic bacteria (ISO 21149:2006)**

Cosmétiques - Microbiologie - Dénombrement et détection
des bactéries aérobies mésophiles (ISO 21149:2006)

Kosmetik - Mikrobiologie - Zählung und Nachweis von
aeroben mesophilen Bakterien (ISO 21149:2006)

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Foreword

The text of ISO 21149:2006 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 21149:2009 by Technical Committee CEN/SS H99 “Products for household and leisure use - Undetermined” the secretariat of which is held by CMC.

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of an identical text or by endorsement, at the latest by December 2009, and conflicting national standards shall be withdrawn at the latest by December 2009.

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Endorsement notice

The text of ISO 21149:2006 has been approved by CEN as a EN ISO 21149:2009 without any modification.

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Cosmetics — Microbiology — Enumeration and detection of aerobic mesophilic bacteria

1 Scope

This International Standard gives general guidelines for enumeration and detection of mesophilic aerobic bacteria present in cosmetics,

- by counting the colonies on agar medium after aerobic incubation, or
- by checking the absence of bacterial growth after enrichment.

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

If needed, microorganisms enumerated or detected may be identified using suitable identification tests described in the standards given in the Bibliography.

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, extreme pH values, etc.

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 21148:2005, *Cosmetics — Microbiology — General instructions for microbiological examination*

3 Terms and definitions

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

3.1

aerobic mesophilic bacteria

mesophilic bacteria growing aerobically under the conditions specified in this International Standard

NOTE In the described conditions, other types of microorganisms (e.g. yeast, mould) can be detected.

3.2

product

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

3.3

sample

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

3.4

initial suspension

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

3.5

sample dilution

dilution of the initial suspension

4 Principle

4.1 General

This method involves enumeration of colonies on a non-selective agar medium or by the presence or absence of bacterial growth after enrichment. The possible inhibition of microbial growth by the sample shall be neutralized to allow the detection of viable microorganism ^[1]. In all cases and whatever the methodology, the neutralization of the antimicrobial properties of the product shall be checked and validated ^{[2] [3] [4]}.

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 $32,5\text{ }^{\circ}\text{C} \pm 2,5\text{ }^{\circ}\text{C}$ for $72\text{ h} \pm 6\text{ h}$.
- Counting the number of colony forming units (CFU) and calculation of the number of aerobic mesophilic bacteria per millilitre or per gram of product.

4.3 Membrane filtration

Membrane filtration consists of the following steps.

- Transfer a suitable amount of the sample prepared as validated in the filtration apparatus wetted with a small volume of an appropriate sterile diluent, filter immediately and wash according to the validated procedure (see 13.3.4). Transfer the membrane filter onto the surface of the specified agar medium as specified in ISO 21148.
- Aerobic incubation of the membranes at $32,5\text{ }^{\circ}\text{C} \pm 2,5\text{ }^{\circ}\text{C}$ for $72\text{ h} \pm 6\text{ h}$.
- Counting the number of colony forming units (CFU) and calculation of the number of aerobic mesophilic bacteria per millilitre or per gram of product.

4.4 Detection of bacteria by enrichment

Detection of bacteria by enrichment consists of the following steps:

- Incubation at $32,5\text{ °C} \pm 2,5\text{ °C}$ for at least 20 h of a defined quantity of the initial suspension in a non-selective liquid medium containing suitable neutralizers and/or dispersing agents.
- Transfer of a defined quantity of the previous suspension on non-selective solid agar medium.
- Aerobic incubation at $32,5\text{ °C} \pm 2,5\text{ °C}$ for 48 h to 72 h.
- Detection of growth and expression of results as “presence/absence” of aerobic mesophilic bacteria per sample *S* of product.

5 Diluents, neutralizers and culture media

5.1 General

General specifications are given in ISO 21148. When water is used 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 and detection of aerobic mesophilic bacteria. 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 specimen to be tested has antimicrobial properties. The efficacy of the neutralization shall be demonstrated before the determination of the count (see Clause 13). Information relative to suitable neutralizers is given in Annex D.

5.2.2 Neutralizing diluents

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	40,0 ml
Water	960,0 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 obtain 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

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 $7,1 \pm 0,2$ 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 the bacterial suspension (tryptone sodium chloride solution)

5.3.1 Composition

Tryptone, pancreatic digest of casein	1,0 g
Sodium chloride	8,5 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 $7,0 \pm 0,2$ 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 instructions of the manufacturer. Ready-to-use media may be used when their composition and/or growth yields are comparable to those of the formulas given herein.

5.4.2 Culture media for counting

5.4.2.1 Soybean–casein digest agar medium (SCDA) or tryptic soy agar (TSA)

5.4.2.1.1 Composition

Pancreatic digest of casein	15,0 g
Papaic digest of soybean meal	5,0 g