

---

---

**Cosmetics — Microbiology —  
Enumeration and detection of aerobic  
mesophilic bacteria**

*Cosmétiques — Microbiologie — Dénombrement et détection des  
bactéries aérobies mésophiles*



This document is a preview generated by EBS



**COPYRIGHT PROTECTED DOCUMENT**

© ISO 2017, Published in Switzerland

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office  
Ch. de Blandonnet 8 • CP 401  
CH-1214 Vernier, Geneva, Switzerland  
Tel. +41 22 749 01 11  
Fax +41 22 749 09 47  
copyright@iso.org  
www.iso.org

# Contents

Page

|                                                                               |           |
|-------------------------------------------------------------------------------|-----------|
| <b>Foreword</b>                                                               | <b>v</b>  |
| <b>1 Scope</b>                                                                | <b>1</b>  |
| <b>2 Normative references</b>                                                 | <b>1</b>  |
| <b>3 Terms and definitions</b>                                                | <b>1</b>  |
| <b>4 Principle</b>                                                            | <b>2</b>  |
| 4.1 General                                                                   | 2         |
| 4.2 Plate count                                                               | 2         |
| 4.3 Membrane filtration                                                       | 2         |
| 4.4 Detection of bacteria by enrichment                                       | 3         |
| <b>5 Diluents, neutralizers and culture media</b>                             | <b>3</b>  |
| 5.1 General                                                                   | 3         |
| 5.2 Neutralizing diluents and diluents                                        | 3         |
| 5.3 Diluent for the bacterial suspension (tryptone sodium chloride solution)  | 4         |
| 5.4 Culture media                                                             | 4         |
| <b>6 Apparatus and glassware</b>                                              | <b>7</b>  |
| <b>7 Strains of microorganisms</b>                                            | <b>7</b>  |
| <b>8 Handling of cosmetic products and laboratory samples</b>                 | <b>7</b>  |
| <b>9 Procedure</b>                                                            | <b>7</b>  |
| 9.1 General recommendation                                                    | 7         |
| 9.2 Preparation of the initial suspension                                     | 7         |
| 9.2.1 General                                                                 | 7         |
| 9.2.2 Water-miscible products                                                 | 8         |
| 9.2.3 Water-immiscible products                                               | 8         |
| 9.3 Counting methods                                                          | 8         |
| 9.3.1 Dilutions for counting methods                                          | 8         |
| 9.3.2 Plate-count methods                                                     | 8         |
| 9.4 Enrichment                                                                | 9         |
| 9.4.1 General                                                                 | 9         |
| 9.4.2 Incubation of the sample                                                | 9         |
| <b>10 Counting of colonies (plate counts and membrane filtration methods)</b> | <b>9</b>  |
| <b>11 Detection of growth (enrichment method)</b>                             | <b>9</b>  |
| <b>12 Expression of results</b>                                               | <b>10</b> |
| 12.1 Method of calculation for plate count                                    | 10        |
| 12.2 Interpretation                                                           | 11        |
| 12.3 Examples                                                                 | 11        |
| 12.4 Detection after enrichment                                               | 13        |
| <b>13 Neutralization of the antimicrobial properties of the product</b>       | <b>13</b> |
| 13.1 General                                                                  | 13        |
| 13.2 Preparation of inoculum                                                  | 14        |
| 13.3 Suitability of counting methods                                          | 14        |
| 13.3.1 Principle                                                              | 14        |
| 13.3.2 Suitability test of the pour-plate method                              | 14        |
| 13.3.3 Suitability of the surface spread method                               | 14        |
| 13.3.4 Suitability of the membrane filtration method                          | 14        |
| 13.4 Suitability of the detection method by enrichment                        | 15        |
| 13.4.1 Procedure                                                              | 15        |
| 13.4.2 Interpretation of results                                              | 15        |
| 13.5 Interpretation of suitability test results                               | 15        |
| <b>14 Test report</b>                                                         | <b>16</b> |

|                                                                                                          |           |
|----------------------------------------------------------------------------------------------------------|-----------|
| <b>Annex A (informative) Other neutralizing diluents</b>                                                 | <b>17</b> |
| <b>Annex B (informative) Other diluents</b>                                                              | <b>19</b> |
| <b>Annex C (informative) Other culture media</b>                                                         | <b>20</b> |
| <b>Annex D (informative) Neutralizers of antimicrobial activity of preservatives and rinsing liquids</b> | <b>23</b> |
| <b>Bibliography</b>                                                                                      | <b>24</b> |

## 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.

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](http://www.iso.org/directives)).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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](http://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 21149:2006), of which it constitutes a minor revision with the following changes:

- in the Scope, “validated” has been changed to “shown to be suitable”;
- in the Scope, “see ISO 29621” has been added and the reference has been added to the Bibliography;
- in [4.1](#), “validated” has been changed to “demonstrated”;
- in [4.3](#), “validated” has been changed to “described”;
- in 5.1, “specifications” has been changed to “instructions”;
- in [9.3.2.1](#), [9.3.2.2](#) and [9.3.2.3](#), “validated” has been changed to “described”;
- in [9.3.2.3](#), “procedure developed during the validation” has been changed to “suitability test procedure”;
- in [9.4.1](#), “validation” has been changed to “suitability test”;
- in [12.2.1](#), “validated according to the chosen method” has been changed to “demonstrated to be suitable for the chosen method”;
- in [13.3](#) and [13.4](#), “validation” has been changed to “suitability”;
- in [13.3.2](#), [13.3.3](#) and [13.3.4](#), “validation” has been changed to “suitability”;
- in [13.3.2](#), [13.3.3](#) and [13.3.4](#), “if the validation count is at least 50 % (0,3 log) of the control count” has been changed to “if the count is at least 50 % of the control”;
- in [13.4.1](#), instances of “validation test” have been changed to “suitability test”;

- in [13.4.2](#), instances of “validation plate” have been changed to “suitability test plate”;
- in [13.5](#), “validation results” has been changed to “suitability test results” and “validation plates” has been changed to “suitability test plates”;
- in [Clause 14](#) f), “validation of the method” has been changed to “demonstration of the suitability”;
- in [A.1](#), [B.1](#) and [C.1](#), “validated” has been changed to “demonstrated to be suitable”.

# Cosmetics — Microbiology — Enumeration and detection of aerobic mesophilic bacteria

## 1 Scope

This document gives general guidelines for enumeration and detection of aerobic mesophilic 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 shown to be suitable.

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 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, hydro-alcoholic products, extreme pH values, etc.

## 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 21148:2017, *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

#### **aerobic mesophilic bacterium**

mesophilic bacterium growing aerobically under the conditions specified in this document

Note 1 to entry: In the described conditions, other types of microorganisms (e.g. yeast, mould) can be detected.