INTERNATIONAL STANDARD

ISO 21148

Second edition
2017-06

Cosmetics — Microbiology — General instructions for microbiological examination

Cosmétiques — Microbiologie — Instructions générales pour les examens microbiologiques

Reference number
ISO 21148:2017(E)

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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).

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).

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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 21148:2005), of which it constitutes a minor revision.

It also incorporates the Technical Corrigendum ISO 21148:2005/Cor 1:2006.

The following changes have been made:

a) in the Introduction, "validated" was changed to "demonstrated to be suitable";

b) in Clause 6, "validation of the methodology" was changed to "verification of the methods' suitability";

c) in 8.2.1, "validated" was changed to "demonstrated to be suitable";

d) in Clause 13, "validated" was changed to "demonstrated";

e) in A.5, "validated" was changed to "demonstrated to be suitable";

f) in B.3, editorial changes were applied.

Introduction

The purpose of this document is to help ensure that the general techniques used for conducting cosmetic microbiological examinations are the same in other laboratories that adopt these standards, to help achieve homogeneous results in different laboratories and to contribute towards the protection of the health of the laboratory personnel by preventing risk of infection.

When conducting microbiological examinations for cosmetic products, it is especially important that:

— only those microorganisms which are present in the samples be isolated or enumerated;

— the microorganisms do not contaminate the environment.

In order to achieve this, it is necessary to pay attention to personal hygiene and to use working techniques which ensure, as far as possible, exclusion of extraneous contamination.

Since, in this document, it is possible to give only a few examples of the precautions to be taken during microbiological examinations, a thorough knowledge of the microbiological techniques and of the microorganisms involved is essential. It is important that the analyses be conducted as accurately as possible, including calculation of the number of microorganisms.

A large number of manipulations can, for example, unintentionally lead to cross-contamination and the analyst should always verify the accuracy of the results given by his/her technique. It is necessary to take special precautions, not only for reasons of hygiene, but also to ensure good reproducibility of the results. It is not possible to specify all the precautions to be taken in all circumstances, but this document at least provides the main measures to be taken when preparing, sterilizing and storing the media and the equipment.

The given recommendations will allow enumeration and detection of mesophilic microorganisms which may grow under aerobic conditions.

The recommendations are applicable to the determination of the absence of, or limited occurrence of specified microorganisms that are of interest for cosmetic products.

The test methods are described in the individual standards. Alternative microbiological procedures can be used provided that their equivalence has been demonstrated or the method has been otherwise demonstrated to be suitable. The choice of a specific method, or combination of methods mentioned in these International Standards will depend on the purpose for performing the test and it is for the user to decide which approach is best for his/her application.

Cosmetics — Microbiology — General instructions for microbiological examination

1 Scope

This document gives general instructions for carrying out microbiological examinations of cosmetic products, in order to ensure their quality and safety, in accordance with an appropriate risk analysis (e.g. low water activity, hydro-alcoholic, extreme pH values).

Because of the large variety of products and potential uses within this field of application, these instructions might not be appropriate for some products in every detail (e.g. certain water-immiscible products).

2 Normative references

There are no normative references in this document.

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

3.2
sample
portion of the *product* (3.1) (at least 1 g or 1 ml) that is used in the test to prepare the *initial suspension* (3.3)

3.3
initial suspension
suspension (or solution) of the *sample* (3.2) in a defined volume of an appropriate enrichment broth

3.4
sample dilution
dilution of the *initial suspension* (3.3)

4 Premises

4.1 Test areas

The areas required for the specific operation of a microbiology laboratory are as follows:

— receipt, storage, preparation and processing of the samples;

— preparation and sterilization of culture media, apparatus and glassware;

— performance of analyses: weighing, dilutions, inoculations, subculturing, incubation, maintenance of the strain, etc.;

— decontamination and cleaning of apparatus, glassware and processing of the analysis waste.

4.2 Additional areas

The areas included in this category are, for example:

— entrances, corridors, stairways, lifts;

— administrative areas (e.g. secretarial, offices, documentation rooms, etc.);

— cloakrooms and toilets;

— archive rooms;

— stores.

4.3 Location of the premises

The environment within which the microbiological analyses are carried out shall not affect the reliability of the analyses.

Care shall be taken to locate the premises so as to avoid risk of cross-contamination.

Care shall be taken to ensure protection against extreme conditions such as excess temperature, dust, humidity, steam, noise, vibration, exposure to direct sunlight, etc.

The surface area shall be sufficiently large to keep the work areas clean and orderly.

During the course of the tests, care shall be taken to limit access to the test areas to only those persons required to conduct the tests.

Separate rooms and/or separate areas and/or specific enclosures should be provided for the following:

— receipt, storage and preparation of samples;

— manipulation of microbial cultures;

— preparation of culture media, apparatus and glassware;

— decontamination and washing area;

— sterilization;

— incubators, refrigerators and freezers.

4.4 Equipping the premises

4.4.1 The test premises shall be fitted out in the following ways in order to reduce the risks of contamination by dust and therefore by microorganisms:

— walls, ceilings and floors should be smooth, non-porous, easy to clean and resistant to detergents and disinfectants used in laboratories;

— overhead pipes conveying fluids should not cross the premises unless they are hermetically enclosed;

— sun-protection systems, when used, shall be installed on the outside of the windows, where practicable;

— windows and doors shall be able to be closed when conducting the test in order to minimize draughts. Furthermore, they shall be designed so as to avoid the formation of dust traps and hence, to facilitate the cleaning.

4.4.2 The ambient temperature and air quality (microorganism content, humidity, dust-spreading rate, etc.) shall be compatible with carrying out the tests.

According to needs, a filter-ventilation and/or a microbiological cabinet are recommended for this purpose.

4.4.3 The laboratory bench tops and furniture shall be made of smooth, non-porous impermeable materials, which are easy to clean and disinfect. Cabinet and equipment tops should be accessible for cleaning.

Non-fixed laboratory furniture shall be designed so as to facilitate cleaning the floors.

It is desirable that documents or books that are not frequently used be kept outside the test areas.

4.5 Maintenance

The floors, walls, ceiling, laboratory bench tops and furniture shall be maintained in good order to avoid cracks where dirt might particularly accumulate and thus cause a source of contamination.

Regular cleaning and, when relevant, disinfection shall be carried out in order to keep the premises in a condition suitable for conducting tests.

The ventilation systems and their filters shall be regularly maintained and filters changed when necessary.

5 Equipment

5.1 General

In general, all equipment shall be kept clean and in proper working condition.

Maintenance operations should be monitored. The measurement instruments and apparatus shall be regularly verified according to an appropriate timetable and results recorded.

5.2 Microbiological cabinets

Cabinets are of two types:

a) clean-air cabinets, which are intended to protect the product from extraneous contamination and to minimize contamination due to the operator;

b) safety cabinets, which are intended to protect the product from extraneous contamination, and also to protect the operator and the environment.

Either cabinet can be used. Safety cabinets should be used for all work involving risk for the operator.

A cabinet is a dust-free workstation equipped with vertical laminar airflow. In microbiology, a safety cabinet is used to retain the microorganisms on filters.

5.3 Balances

A microbiology laboratory for analyses of cosmetic products should be equipped with balances of the required range and accuracy for the different products to be weighed. Generally, the accuracy required

for weighing the samples to be analysed and some components of the culture media and reagents is ±0,01 g.

5.4 Homogenizer

This equipment (e.g. blender, stomacher, etc.) may be used to prepare the initial suspension from the test samples of non-liquid products.

5.5 pH–meter

The pH-meter should be capable of measuring to an accuracy of ±0,1 pH units and its minimum measuring threshold shall be 0,01 pH units.

5.6 Autoclave

The autoclave shall be kept in good operating condition and shall regularly be inspected by the competent departments in accordance with the manufacturer's instructions and proper documentation should be recorded.

The autoclave shall not be used to sterilize both clean materials and also to decontaminate used materials at the same time. Wherever possible, separate autoclaves for these two processes should be used.

5.7 Incubator

Incubators shall be equipped with a regulation system which allows the temperature to be kept even and stable over their entire working volume.

If the ambient temperature is close to, or higher than, that of the incubator, use an incubator with a cooling system.

Incubators should be protected from direct sunlight.

If possible, incubators should not be completely filled in one single operation because the culture media will take a long time to equilibrate to temperature, whatever type of incubator is used (forced-air convection or otherwise).

The temperature shall be checked and recorded at least every working day.

5.8 Water baths

Water baths are of two types:

— thermostatically-controlled baths, suitable for incubation of inoculated culture media, for identification tests, etc.;

— temperature-controlled water baths for maintenance of sterile agar media in a molten state for later use in specified procedures.

The required temperature and accuracy are stipulated in each method of application.

5.9 Refrigerator or cold-storage room

The temperature, unless otherwise specified, shall be 5 °C ± 3 °C.

5.10 Freezer

The temperature, unless otherwise specified, shall be below –18 °C.

5.11 Sterilizing oven

A sterilizing oven is a chamber which allows the destruction of microorganisms by dry heat.

The temperature shall be evenly distributed within the chamber.

The oven shall be equipped with:

— a thermostat;

— a thermometer or a recording thermocouple;

— a duration indicator or a programmer/timer.

5.12 Colony-counting device

A colony-counting device may be used.

5.13 Other equipment

WARNING — Volumetric glassware shall not be sterilized in a sterilizing oven.

Other equipment and apparatus for everyday use include the following:

a) filtration apparatus (see below);

b) glass or plastic containers (test tubes, flasks, bottles);

c) glass or plastic Petri dishes (most commonly between 85 mm and 100 mm in diameter);

d) glass or plastic pipettes (10 ml, 2 ml, 1 ml), automatic pipettes;

e) sampling instruments;

f) wires and loops (of nickel/chromium, platinum/iridium or disposable plastic, etc.);

g) optical microscope;

h) gas burner or wire incinerator;

i) dispenser for culture media and reagents;

j) mechanical stirrer.

If the membrane filtration method is used, the equipment shall also include:

— a membrane filtration system or filtration apparatus constructed of a suitable material, with a filter holder of at least 50 ml, and suitable for use of filters with diameter 47 mm to 50 mm and not more than 0,45 µm pore size;

— the type of membrane material is chosen in such a way that the bacteria are not affected by the residual components of the sample to be investigated;

— a vacuum source able to give an even filtration flow rate (the device shall be set as to obtain the filtration of 100 ml of liquid in less than 2 min).

6 Strains of microorganisms

The strains needed for the verification of the methods' suitability are indicated in each method of application.