INTERNATIONAL STANDARD

ISO 18415

First edition
2007-09-01

Corrected version
2007-12-01

Cosmetics — Microbiology — Detection of specified and non-specified microorganisms

Cosmétiques — Microbiologie — Détection des micro-organismes spécifiés et non spécifiés

Reference number
ISO 18415:2007(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Contents

Page

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.

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.

ISO 18415 was prepared by Technical Committee ISO/TC 217, *Cosmetics*.

This corrected version of ISO 18415:2007 contains the following corrections:

— p. 2, 3.6.1: modification of definition;

— p. 3, 3.8: correction of term's number;

— p.8, 9.7.1: correction of text in the second paragraph.

Introduction

Microbiological examinations of cosmetic products are carried out according to an appropriate microbiological risk analysis in order to ensure their quality and safety for consumers.

Microbiological risk analysis depends on several parameters such as:

— potential alteration of cosmetic products;

— pathogenicity of microorganisms;

— site of application of the cosmetic product (hair, skin, eyes, mucous membranes);

— type of user (adults, children, including under 3 years).

For cosmetics and other topical products, the detection of skin pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* may be relevant because they can cause skin or eye infection. The detection of other kinds of microorganisms might be of interest since these microorganisms (including indicators of faecal contamination e.g. *Escherichia coli*) suggest hygienic failure during manufacturing process.

Cosmetics — Microbiology — Detection of specified and non-specified microorganisms

1 Scope

This International Standard gives general guidelines for the detection and identification of specified microorganisms in cosmetic products as well as for the detection and identification of other kinds of aerobic mesophilic non-specified microorganisms in cosmetic products.

Microorganisms considered as specified in this International Standard might differ from country to country according to national practices or regulations. Most of them considered as specified microorganisms include one or more of the following species: *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* and *Candida albicans*.

In order to ensure product quality and safety for consumers, it is advisable to perform an appropriate microbiological risk analysis in order to determine the types of cosmetic product 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.

The method described in this International Standard is based on the detection of microbial growth in a non-selective liquid medium (enrichment broth) suitable to detect microbial contamination, followed by isolation of microorganisms on non-selective agar media. Other methods can be appropriate depending on the level of detection required.

In this International Standard specific indications are given for identification of *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* and *Candida albicans*. Other microorganisms that grow under the conditions described in this International Standard, may be identified by using suitable tests according to a general scheme (see Annex A). Other standards (e.g., ISO 18416, ISO 21150, ISO 22717, ISO 22718) may be appropriate.

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

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*

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

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

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

3.4
sample dilution
dilution of the initial suspension

3.5
aerobic mesophilic microorganisms
mesophilic bacteria or yeast growing aerobically under the conditions specified in this International Standard

NOTE In the described conditions other types of microorganism (e.g. molds) are detectable.

3.6
specified microorganisms
aerobic mesophilic bacteria or yeast undesirable in a cosmetic product and recognised as a skin pathogen species that may be harmful for human health or as an indication of hygienic failure in the manufacturing process

3.6.1
Pseudomonas aeruginosa
Gram-negative rod (bacilli), motile, smooth colonies pigmented brown or greenish

NOTE 1 The main characteristics for identification are growth on a selective cetrimide agar medium, oxidase positive, production of diffusible fluorescent pigments and production of a soluble phenazine pigment (pyocyanin) in suitable media.

NOTE 2 *Pseudomonas aeruginosa* can be isolated from a wide variety of environmental sources, especially in water and has a very high potential to spoil many different substrates. It can produce infections of human skin or eye areas. It is undesirable in cosmetic products for its potential pathogenicity and its capacity to affect the physico-chemical properties of the cosmetic formula.

3.6.2
Escherichia coli
Gram-negative rod (bacilli), motile, smooth colonies

NOTE 1 The main characteristics are catalase positive, oxidase negative, fermentation of lactose, production of indole, growth on selective medium containing bile salts with characteristic colonies.

NOTE 2 *Escherichia coli can* be isolated from the moist environmental sources (air, water, soil) and is a faecal contamination indicator.

3.6.3
Staphylococus aureus
Gram-positive cocci, mainly aggregated in grape-like clusters, smooth colonies generally pigmented in yellow

NOTE 1 The main characteristics for identification are growth on a specific selective medium, catalase positive, coagulase positive.

NOTE 2 *Staphylococcus aureus* is an opportunistic pathogen for humans, which often can be also present on the skin of healthy individuals without causing them any apparent illness. It is a specified microorganism and undesirable in cosmetic products.

3.6.4
Candida albicans
yeast that forms white to beige, creamy and convex colonies on the surface of a non-selective agar medium

NOTE The main characteristics for identification are production of germ tube and/or pseudomycelium and chlamydospore when the test is performed following the method specified in this International Standard

3.7
non-specified microorganism
aerobic mesophilic bacteria or yeast found in cosmetic products, not defined in 3.6

3.8
enrichment broth
non-selective liquid medium containing suitable neutralizers and/or dispersing agents and validated for the product under test

4 Principle

The first step of the procedure is to perform an enrichment by using a non-selective broth medium to increase the number of microorganisms without the risk of inhibition by the selective ingredients that are present in selective/differential growth media.

The following steps (isolation and identification) are performed according to need by using appropriate conditions of incubation and suitable identification test, as described in this International Standard.

The possible inhibition of microbial growth by the sample shall be neutralized to allow the detection of viable microorganisms [2]. In all cases and whatever the methodology, the neutralization of the antimicrobial properties of the product shall be checked and validated [2],[3],[4].

5 Diluents 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 enrichment broth is used to disperse the sample and to increase the initial microbial population. It may contain neutralizers if the specimen to be tested has antimicrobial properties. The efficacy of the neutralization shall be demonstrated (see Clause 11). Information relative to suitable neutralizers is given in Annex C.

The enrichment broth (5.3.2.1) or any of the ones listed in Annex B is suitable for checking the presence of specified and non-specified microorganisms in accordance with this International Standard provided that they are validated in accordance with Clause 11.

Other diluents and culture media may be used if it has been demonstrated that they are suitable for use.

5.2 Diluent for the microbial suspension (tryptone sodium chloride solution)

5.2.1 General

The diluent is used for the preparation of bacteria and yeast suspensions used for the validation procedure (see Clause 11).

5.2.2 Composition

— tryptone, pancreatic digest of casein 1,0 g

— sodium chloride 8,5 g

— water 1 000 ml

5.2.3 Preparation

Dissolve the components in 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.

5.3 Culture media

5.3.1 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.

5.3.2 Enrichment broth

5.3.2.1 Eugon LT100 broth

5.3.2.1.1 General

This medium contains ingredients which neutralize inhibitory substances present in the sample: lecithin and polysorbate 80, and dispersing agent: octoxynol 9.

5.3.2.1.2 Composition

— pancreatic digest of casein 15,0 g

— papaic digest of soybean meal 5,0 g

— *L*-cystine 0,7 g

— sodium chloride 4,0 g

— sodium sulfite 0,2 g

— glucose 5,5 g

— egg lecithin 1,0 g

— polysorbate 80 5,0 g

— octoxynol 9 1,0 g

— water 1 000 ml

5.3.2.1.3 Preparation

Dissolve the components polysorbate 80, octoxynol 9 and egg lecithin, one after another in boiling water to complete dissolution. Dissolve the other components by mixing while heating. Dispense the medium into suitable containers. Sterilize in the autoclave at 121 °C for 15 min. After sterilization, the pH shall be equivalent to 7,0 ± 0,2 when measured at room temperature.

5.3.2.2 Other enrichment broths

Other enrichment broths may be used as appropriate (see Annex B).

5.3.3 Non-selective agar medium

5.3.3.1 General

This medium is used for the isolation and detection of specified and non-specified microorganisms present in the initial suspension after enrichment and for the preparation of inoculum used in the validation procedure.

5.3.3.2 Soybean-casein digest agar medium (SCDA) or tryptic soy agar (TSA)

5.3.3.2.1 Composition

— pancreatic digest of casein 15,0 g

— papaic digest of soybean meal 5,0 g

— sodium chloride 5,0 g

— agar 15,0 g

— water 1 000 ml

5.3.3.2.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by mixing while heating. Dispense the medium into suitable containers. Sterilize in the autoclave at 121 °C for 15 min. After sterilization and cooling down, the pH shall be equivalent to 7,3 ± 0,2 when measured at room temperature.

5.3.3.3 Other non-selective agar medium

Other non-selective, non-neutralizing agar media may be used (see Annex B).

6 Apparatus and glassware

The laboratory equipment, apparatus and glassware are described in ISO 21148.

7 Strains of microorganism

For testing the recovery efficiency of the test conditions, three specified microorganisms are used: two strains representative of both Gram-negative and Gram-positive bacteria and a strain of yeast.

— *Pseudomonas aeruginosa* ATCC 9027 (equivalent strain: CIP 82.118 or NCIMB 8626 or NBRC 13275 or KCTC 2513 or other equivalent national collection strain).