
**Cosmetics — Microbiology —
Detection of *Escherichia coli***

Cosmétiques — Microbiologie — Détection d'Escherichia coli

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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.

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).

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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 meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](#)

The committee responsible for this document is ISO/TC 217, *Cosmetics*.

This second edition cancels and replaces the first edition (ISO 21150:2006), which has been technically revised.

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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 the following:

- potential alteration of cosmetic products;
- pathogenicity of microorganisms;
- site of application of the cosmetic product (hair, skin, eyes, mucous membranes);
- type of users (adults, children 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 infections. The detection of other kinds of microorganism might be of interest since these microorganisms (including indicators of faecal contamination e.g. *Escherichia coli*) suggest hygienic failure during the manufacturing process.

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Cosmetics — Microbiology — Detection of *Escherichia coli*

1 Scope

This International Standard gives general guidelines for the detection and identification of the specified microorganism *Escherichia coli* in cosmetic products. Microorganisms considered as specified in this International Standard might differ from country to country according to national practices or regulations.

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 (see ISO 29621) 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 *Escherichia coli* in a non-selective liquid medium (enrichment broth), followed by isolation on a selective agar medium. Other methods may be appropriate, depending on the level of detection required.

NOTE For the detection of *Escherichia coli*, subcultures can be performed on non-selective culture media followed by suitable identification steps (e.g. using identification kits).

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 International Standards (ISO 18415) may be appropriate. 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 shown to be suitable.

2 Normative references

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The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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 (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.

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

specified microorganism

aerobic mesophilic bacterium or yeast that is undesirable in a cosmetic product and is recognized 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

Escherichia coli

gram-negative rod, motile, smooth colonies

Note 1 to entry: The main characteristics for identification are catalase positive, oxidase negative, fermentation of lactose, production of indole, growth on selective medium containing bile salts with characteristic colonies.

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

3.7

enrichment broth

non-selective liquid medium containing suitable neutralizers and/or dispersing agents and demonstrated to be suitable for the product under test

4 Principle

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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 second step of the test (isolation) of the test is performed on a selective medium followed by identification tests.

The possible inhibition of microbial growth by the sample shall be neutralized to allow the detection of viable microorganisms.^[1] In all cases and whatever the methodology, the neutralization of the antimicrobial properties of the product shall be checked and demonstrated (see [Clause 11](#)).

5 Diluents and culture media

5.1 General

General instructions are given in ISO 21148. When water is mentioned in this International Standard, 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 B](#).

The enrichment broth ([5.3.3.1](#)) or any of the ones listed in [Annex A](#) is suitable for checking the presence of *Escherichia coli* in accordance with this International Standard provided that they have been demonstrated to be suitable 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 bacterial suspension (tryptone sodium chloride solution)

5.2.1 General

The diluent is used for the preparation of bacterial suspension used for the suitability test 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 and cooling down, the pH shall be equivalent to $7,0 \pm 0,2$ when measured at room temperature.

5.3 Culture media

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5.3.1 General

Culture media may be prepared using the descriptions provided below or from dehydrated culture media, according to the instructions from the manufacturer. The instructions provided by the supplier of the media should be followed. <https://standards.iteh.ai/catalog/standards/sist/07c1e1b-7129-479c-85e2-2637dec2f7f1/iso-21150-2015>

NOTE Ready-to-use media can be used when their composition and/or growth yields are comparable to those of the formulae given herein.

5.3.2 Agar medium for the suitability test (see [Clause 11](#)) [soybean-casein digest agar medium (SCDA) or tryptic soy agar (TSA)]

5.3.2.1 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

5.3.2.2 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 $7,3 \pm 0,2$ when measured at room temperature.

5.3.3 Enrichment broth

5.3.3.1 Eugon LT 100 broth

5.3.3.1.1 General

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

5.3.3.1.2 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

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5.3.3.1.3 Preparation

Dissolve the components, polysorbate 80, octoxynol 9 and egg lecithin, successively into boiling water until their 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 and cooling down, the pH shall be equivalent to $7,0 \pm 0,2$ when measured at room temperature.

5.3.3.2 Other enrichment broths

Other enrichment broths may be used as appropriate (see [Annex A](#)).

5.3.4 Selective agar medium for isolation of *Escherichia coli*

5.3.4.1 MacConkey agar medium

5.3.4.1.1 Composition

— pancreatic digest of gelatin	17,0 g
— pancreatic digest of casein	1,5 g
— peptic digest of animal tissue	1,5 g
— lactose	10,0 g
— bile salts mixture	1,5 g

— sodium chloride	5,0 g
— agar	13,5 g
— neutral red	30,0 mg
— crystal violet	1,0 mg
— water	1 000 ml

5.3.4.1.2 Preparation

Dissolve all solid components in the water and boil for 1 min to effect solution.

Dispense in suitable containers and sterilize at 121 °C for 15 min.

The pH, after sterilization and cooling down, shall be equivalent to $7,1 \pm 0,2$ when measured at room temperature.

5.3.5 Selective agar medium for confirmation of *Escherichia coli*

5.3.5.1 Levine eosin-methylene blue agar medium

5.3.5.1.1 Composition

— pancreatic digest of gelatin	10,0 g
— potassium hydrogen phosphate (K ₂ HPO ₄)	2,0 g
— agar	15,0 g
— lactose	10,0 g
— eosin Y	400 mg
— methylene blue	65 mg
— water	1 000 ml

5.3.5.1.2 Preparation

Dissolve the pancreatic digest of gelatin, the potassium hydrogen phosphate, and the agar in the water, with warming, and allow to cool. Just prior to use, liquefy the gelled agar solution, add the remaining ingredients, as solutions in the following amounts, and mix; for each 100 ml of the liquefied agar solution

- 5 ml of 20 % lactose solution,
- 2 ml of 2 % eosin Y solution, and
- 2 ml of 0,033 % methylene blue solution.

The finished medium may not be clear.

Dispense in suitable containers and sterilize at 121 °C for 15 min.

The pH, after sterilization and cooling down, shall be equivalent to $7,1 \pm 0,2$ when measured at room temperature.