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Cosmetics — Microbiology — Quality control of culture media and diluents used in cosmetics standards

Cosmétiques — Microbiologie — Contrôle qualité des milieux de culture et des diluants utilisés dans les normes relatives aux cosmétiques

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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Foreword

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This document was prepared by Technical Committee ISO/TC 217, *Cosmetics,* in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 392, *Cosmetics,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The quality of culture media used in the current standards for cosmetic microbiology is an essential part of microbiological analysis reliability and needs to be verified.

Checking different parameters of culture media such as growth promotion, absence of microbial growth for non-inoculated culture media, physical characteristics, can help to assess their quality.

This document is intended to provide methods to assess the quality of the media used in cosmetics microbiology standards and define the minimum acceptance criteria required to ensure their performance.

This applies to:

a) commercially ready-to-use culture media;

b) culture media prepared from dehydrated culture media plus additional ingredients, or only ingredients.

Cosmetics — Microbiology — Quality control of culture media and diluents used in cosmetics standards

1 Scope

This document specifies the minimum requirements for quality control of microbiological culture media and diluents in order to demonstrate their ability to detect microorganisms and to ensure reliability of the microbiological test methods described in the ISO cosmetics microbiology standards.

This document describes mainly growth promotion and microbial control tests and is applicable to both commercially ready-to-use culture media and culture media prepared from dehydrated culture media or basic constituents in the user's laboratory.

Other methods can be substituted provided that their equivalence has been demonstrated.

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, *Cosmetics — Microbiology — General instructions for microbiological examination*

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:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
culture medium
mixture of ingredients, in liquid or solid form, prepared according to a formula and intended to support the growth of microorganisms under specific conditions

Note 1 to entry: There are different types of culture media suitable for growing different types of microorganisms depending on different included nutrients and chemicals present in the formulation.

3.1.1
batch of culture medium
lot of culture medium
homogenous and fully traceable unit of culture medium referring to a defined amount of bulk, which has been produced within one defined production period, having been assigned the same batch number

3.1.2
ready-to-use culture medium
sterile *liquid culture medium* (3.1.3) or *solid culture medium* (3.1.4) that is supplied in plates, tubes, or other containers in ready-to-use form

3.1.3
liquid culture medium
culture medium (3.1) consisting in aqueous solution of one or more constituents, such as peptone water or nutrient broth

Note 1 to entry: Liquid culture media in tubes, flasks or bottles are commonly called “broths”.

Note 2 to entry: Enrichment culture media are generally liquid media which, due to their composition, provide favourable conditions for microorganisms’ multiplication.

3.1.4
solid culture medium
culture medium (3.1) containing solidifying substances (e.g. agar, gelatin) in different concentrations

3.1.5
selective culture medium
liquid culture medium (3.1.3) or *solid culture medium* (3.1.4) which allows specifically the growth of a selected microorganism while inhibiting partially or totally the growth of different, non-target microorganisms which can be in the product to be tested

Note 1 to entry: It may have indicative properties with growth of characteristic aspect of colonies if this is a solid culture medium.

3.2
diluent
liquid phase designed to separate microorganisms from a *solid culture medium* (3.1.4) and/or to reduce their concentration by dilution without multiplication or inhibition during the time of contact

Note 1 to entry: Diluent can contain neutralizing agent to inactivate the antimicrobial properties of the product.

3.3
strain
test microorganism used for quality control of the *culture medium* (3.1)

3.3.1
reference strain
test microorganism provided by a reference culture collection centre

3.3.2
reference stock culture
stored reference strain
set of separate identical cultures obtained by a single subculture from the *reference strain* (3.3.1)

Note 1 to entry: The reference stock or stored reference strain can be stored in a seed lot system (e.g. single-use vial or bead) to maintain reference strains in the laboratory.

3.3.3
stock culture
subculture from a *reference stock culture* (3.3.2)

3.3.4
working culture
subculture from a *reference stock culture* (3.3.2) or *stock culture* (3.3.3) and is often kept as slants or plates, used for preparation of calibrated microbial suspension

3.3.5
subculture
passage, i.e. transfer of organisms from a viable culture to fresh medium with growth of the microorganisms

Note 1 to entry: Any form of subculturing is considered to be a transfer/passage.

4 Principle

4.1 General information

The quality control of culture media refers to different parameters such as:

— pH;

— absence of microbial growth;

— growth promotion;

— selective and indicative properties (when relevant).

These are the key parameters to ensure and control the quality of the culture media. However, particular attention should also be paid to:

— the manufacturer's instructions;

— preparing conditions (volume, weighing, water quality);

— sterilization conditions (cycle time, temperature, pressure, packaging);

— storage conditions (temperature, duration).

Failure to comply with these instructions and conditions can affect appearance and functional characteristics provided in the manufacturer's guidance such as colour, gel consistency, clarity and homogeneity.

NOTE For sterilization conditions and/or other conditions, see ISO 21148 and ISO 11133.

The user's laboratory should ensure their own preparation process is accurate.

4.2 pH

pH is an essential physical parameter of all culture media.

The target pH value should be reached after autoclaving when culture media are prepared from dehydrated media or basic constituents in the user's laboratory.

4.3 Absence of microbiological growth

The purpose of this test is to check that the medium does not contain any microbiological contamination which can interfere with microbial tests results.

4.4 Growth promotion

The purpose of this test is to ensure the ability of microorganisms to grow on the culture media.

NOTE Growth promotion is also called 'productivity' of culture medium.

4.5 Selective and indicative properties

The purpose of this test is to verify the ability of the culture medium to allow the growth of target microorganisms and/or to confirm its colony morphology within the range of incubation time and temperature.

An additional purpose of this test is to ensure that there is no growth of the target inhibited microorganism(s).

5 Diluents, neutralizers and culture media

5.1 General

The diluents, neutralizers and culture media suitable for enumeration and detection of microorganisms are described in ISO 11930, ISO 16212, ISO 18415, ISO 18416, ISO 21149, ISO 21150, ISO 21322, ISO 22717 and ISO 22718. Other diluents, neutralizers and culture media may be used if they have been demonstrated to be suitable for use.

Use the general instructions given in ISO 21148. When water is mentioned in this international standard, use water as specified in ISO 21148.

5.2 Diluents and neutralizers

Diluents may be used for preparation and dilutions of calibrated microbial suspensions and to disperse the samples. In this case, it is required that a diluent contains neutralizers if the sample to be tested has antimicrobial properties or contains preservatives. Diluents and neutralizers are described in ISO 11930, ISO 16212, ISO 18415, ISO 18416, ISO 21149, ISO 21150, ISO 21322, ISO 22717 and ISO 22718..

5.3 Culture media

Culture media for enumeration and/or detection of microorganisms are described in ISO 11930, ISO 16212, ISO 18415, ISO 18416, ISO 21149, ISO 21150, ISO 21322, ISO 22717 and ISO 22718.

Culture media may be prepared from dehydrated culture media using specific standards instructions or the instructions provided by the manufacturer of the culture media.

Already prepared commercially manufactured ready-to-use culture media in liquid or solid form may also be used if they meet the criteria described in this document.

6 Apparatus and glassware

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

7 Strains of microorganisms

The culture should be reconstituted according to the procedures provided by the supplier of the reference strain. The strains may be stored as described in EN 12353 or according to another suitable method.

The following strains are used for the growth promotion test. The relationship between the medium to be checked and the test microorganisms to be used is described in Annex A.

— *Pseudomonas aeruginosa* ATCC®9027™[1] (equivalent strain: CIP®82.118™[2] or NCIMB®8626™[3] or NBRC®13275™[4] or KCTC®2513™[5] or WDCM 00026[6] or other equivalent national collection strain);

— *Staphylococcus aureus* ATCC®6538™ (equivalent strain: CIP®4.83™ or NCIMB®9518™ or NBRC®13276™ or KCTC®1916™ or NCTC®10788™[7] or WDCM 00032 or other equivalent national collection strain);

— *Escherichia coli* ATCC®8739™ (equivalent strain: CIP®53.126™ or NCIMB®8545™ or NBRC®3972™ or KCTC®2571™ or NCTC®12923™ or WDCM 00012 or other equivalent national collection strain);

— *Candida albicans* ATCC®10231™ (equivalent strain: IP 48.72™ or NCPF® 3179™[8] or NBRC®1594™ or KCTC®7965™ or WDCM 00054 or other equivalent national collection strain);

— *Aspergillus brasiliensis* ATCC®16404™ (equivalent strain: IP 1431 or IMI®149007™[9] or NBRC®9455™ or KCTC®6196™ or WDCM 00053 or other equivalent national collection strain

Other relevant strains or in-house isolates can be used as needed to supplement the strains cited in this clause.

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