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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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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 document 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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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 culture or stored reference strain can be stored in a seed lot system (e.g. single-use vials or beads) 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 its 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.

1) ATCC®6538™, 9027™, 8739™, 10231™ and 16404™ are trademarks of the American Type Culture Collection. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

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3) NCIMB®8626™, 9518™ and 8545™ are trademarks of the National Collection of Industrial Marine Bacteria. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

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6) WDCM: World Data Center for Microorganisms.

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8) NCPF® 3179™ is a trademark of the National Collection of Pathogenic Fungi. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

9) IMI®149007™ is a trademark of the International Mycological Institute, UK. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

8 Procedure

8.1 General recommendation

For culture media prepared from dehydrated culture media or basic constituents in the users' laboratories, follow the general instructions given in ISO 21148. The supplier's certificate of analysis shall be checked to ensure it complies with specification.

For commercially ready-to-use culture media, the supplier's certificate of analysis shall be checked to ensure it complies with specification.

It is recommended to conduct the following tests for each batch of culture media.

— pH

For culture media prepared from dehydrated media or basic constituents in the users' laboratories, pH shall be measured in accordance with ISO 21148.

— Absence of microbial growth

For checking the absence of microbial growth of the culture media, a fraction of culture media is incubated for specified time and temperatures.

— Growth promotion test

For performing a growth promotion test, a fraction of culture media is inoculated with a known level of microorganisms.

After incubation for specified time and temperatures:

— for solid culture media, the colonies are counted and compared with the theoretical inoculum and/or a control conducted with a previously approved batch.

— for liquid culture media, a clearly visible growth (turbidity, grain, flocculation, etc.), is observed and compared with a negative control (non-inoculated media) and/or a control conducted with a previously approved inoculated batch.

Each culture medium is tested with only one microorganism at a time.

The test microorganisms recommended are listed in Table A.1. They also may be selected from the culture medium manufacturer's recommendation or may include representative environmental isolates.

— Selective and indicative properties

In addition to the properties of growth promotion, qualitative assay can be performed to assess selective properties by inoculating with the target and non-target microorganisms. Growth of the target organism with the absence of the non-target organism should be observed.

Indicative properties are assessed by the morphology of the target microorganism after incubation at specified time and temperature.

8.2 Preparation of strains

8.2.1 General

Commercially prepared strains can be used for microbiological control of the performance of culture media.

The test micro-organisms to be used are listed in Annex A.

To perform the tests, use the strains stored in the laboratory to obtain stock cultures and the working cultures.

The stock culture is a confluent culture obtained by streaking slant tubes or plates with the stored strain. After incubation, the stock culture can be kept between 2 °C and 8 °C for up to two months depending on the microorganisms.

The use of stock culture is optional.

The working culture, prepared when needed to perform test, is used to obtain a calibrated suspension of known microbial content (inoculum).

Microorganisms should be used within five passages of the reference culture.

The same growth conditions (agar media, temperature, and incubation time) are used for both stock cultures and working cultures (see 8.2.2 and 8.2.3).

The preparation of test microorganisms shall be in accordance with Figure 1.

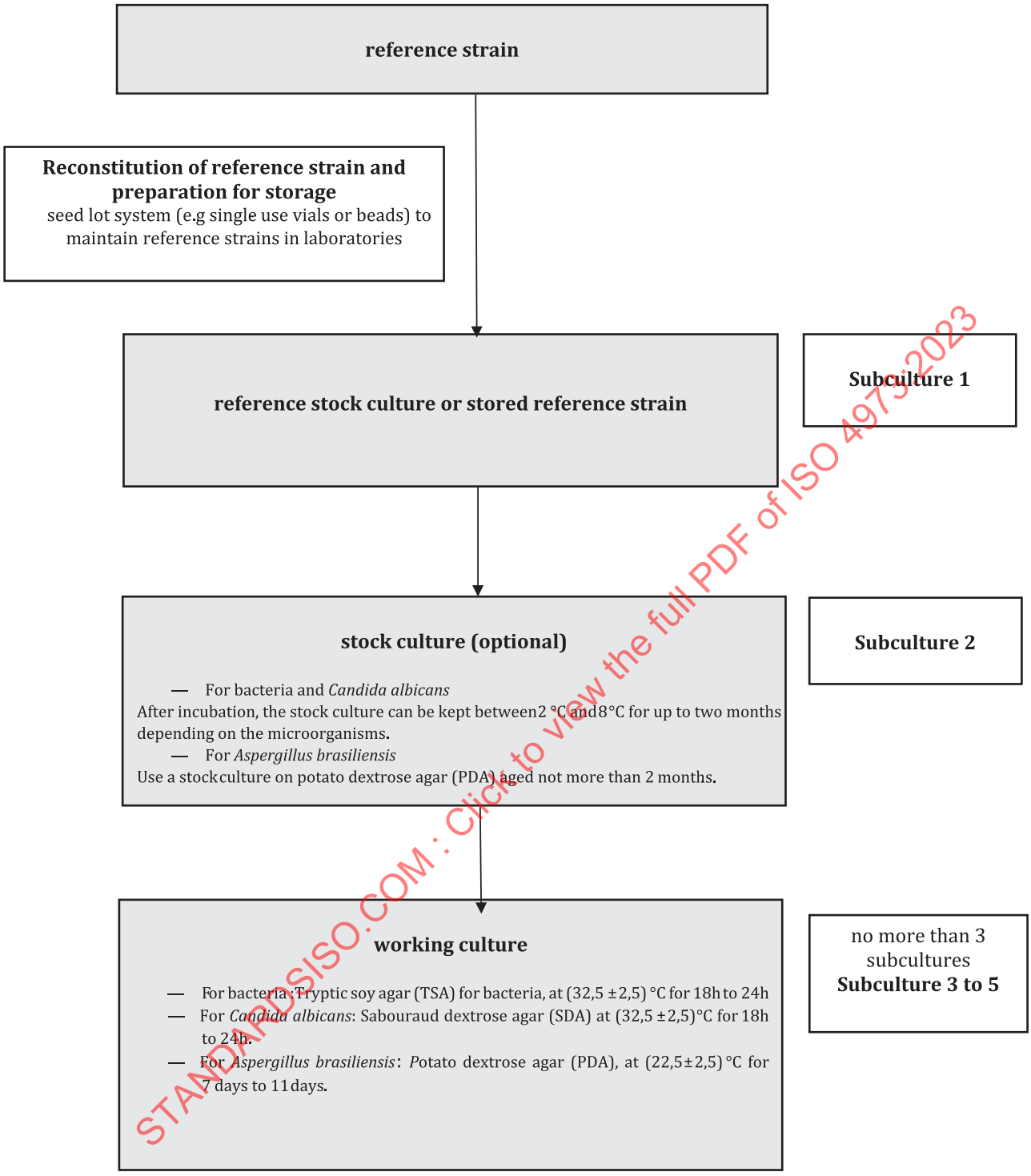

Figure 1 — Flow chart corresponding to the preparation of test microorganisms

NOTE 1 A limited number of serial subcultures and the use of confluent cultures instead of isolated colonies decrease the risk of change in the susceptibility of strains. The standardization of growth conditions and of inoculum preparation improves the reproducibility of the test.

NOTE 2 Changes in susceptibility of strains stored by freezing can be observed (due to repeated heat shocks) when multidose containers are used (for example, containers with several beads brought out the freezer to take one bead, then replaced in the freezer).

NOTE 3 Other culture media can be used according to recommendations of the strains reference culture collection centre.

NOTE 4 Calibrated, commercially prepared, ready-for-use, strains can be used for microbiological control of the performance of culture media.

8.2.2 Preparation of bacterial and *Candida albicans* suspensions

To prepare the working culture of the test microorganism, prepare a subculture from the stock culture by spreading and growing on slant tubes or plates [tryptic soy agar (TSA) for bacteria, Sabouraud dextrose agar (SDA) for Candida albicans].

Incubate at (32,5 ± 2,5) °C for 18 h to 24 h.

It is possible to prepare in the same way a second subculture, starting from the first, and incubate at (32,5 ± 2,5) °C for 18 h to 24 h. A third subculture can be grown in the same way, starting from the second. The second and the third one (if it was carried out) form the working culture. If the second subculture cannot be carried out in a timely manner, then the first subculture can be kept for up to 48 h in the incubator (32,5 ± 2,5) °C and used to prepare the second subculture. In this case, prepare the third 18 h to 24 h subculture and use in the test.

Take 10 ml of diluent for bacterial or yeast suspension and place in a suitable sterile container. Transfer loopfuls of the cells harvested from the agar medium into the diluent; the cells should be suspended in the diluent by rubbing the loop in a small amount of the diluent against the side of the container to dislodge the cells.

NOTE Five grams of sterile glass beads can be added in the suitable sterile container.

Shake the container manually or mechanically, for a maximum of 3 min, to harmonize the suspension. Aspirate the upper part of the suspension (avoiding any contact with the glass beads) and transfer the obtained suspension to a sterile container.

Adjust the number of cells in the suspension about $1x10^3$ CFU/ml (CFU: colony forming unit) for bacteria and *Candida albicans* using the diluent for bacterial and yeast suspension and in accordance with calibration data produced in the laboratory (e.g. using a spectrophotometer, see ISO 21148:2017, Annex C).

Use this calibrated suspension within 2 h or within 24 h if stored at 2 °C to 8 °C.

8.2.3 Preparation of *Aspergillus brasiliensis* spore stock suspension

To obtain the working culture of the test microorganism, use a stock culture on potato dextrose agar (PDA) aged not more than 2 months, and prepare a suspension in the diluent for preparation of *Aspergillus brasiliensis* spore suspension. Inoculate by flooding the surface of PDA, in a Roux flask (or an appropriate number of Petri dishes), so as to obtain a confluent culture. Incubate at (22,5 ± 2,5) °C for 7 days to 11 days.

After incubation, transfer 10 ml of the polysorbate solution on to the PDA surface. Gently detach the spores from the culture surface, for example using a spatula or glass beads.

Transfer the suspension to an appropriate flask and stir gently for about 1 min in the presence of glass beads. Filter the suspension through a sintered filter of porosity 2 (i.e. 40 µm to 100 µm).

Carry out a microscopic examination (magnification x400) to detect the presence of germinated spores or mycelium fragments.

— If germinated spores are present, the suspension shall be discarded.

— If mycelium is present in more than one field out of ten, the suspension shall be discarded. However, it is possible to wash the filtered suspension by centrifuging at 2 000 g for 20 min. Wash the spores at least twice by re-suspending them in the polysorbate solution and centrifuging and carry out a microscopic examination (magnification x400) to check the efficacy of the washing/centrifugation.

Adjust the number of spores in the suspension to the value of about $1x10^3$ spores/ml using the diluent for preparation of *Aspergillus brasiliensis* spore suspension and any appropriate means.

The use of a cell enumeration device (e.g. a haemocytometer) is recommended to adjust the number of spores. If an appropriate cell count chamber is used, follow the instructions accurately.

The suspension should be used during the same working day or the following day if stored between 2 °C and 8 °C. For longer period of storage, the stable spore suspension may be maintained at 2 °C to 8 °C for a validated period of time (absence of germinated spores).

8.2.4 Control of the concentration of the calibrated suspension

Check the initial concentration of the calibrated suspension.

— Make successive tenfold dilutions of the calibrated suspension in the appropriate diluent.

— The number of dilutions of the calibrated suspension prepared in 8.2 depends on the used method: pour plate or surface spread method.

— Perform in duplicate the enumeration of 1 ml of the suitable dilution:

  — into TSA for bacteria and into SDA for *Candida albicans*. Incubate the dishes at (32,5 ± 2,5) °C for 24 h to 48 h;

  — into SDA for *Aspergillus brasiliensis*. Incubate the dishes at (22,5 ± 2,5) °C for 3 days to 5 days.

8.3 Absence of microbial growth

8.3.1 Solid culture media

Incubate a fraction of the culture media at the same time and temperature and for the longest duration as the tested microorganisms.

8.3.2 Liquid culture media and diluents

Incubate a fraction of the culture media and/or diluents at the same time and temperature and for the longest duration as the tested microorganisms.

8.4 Growth promotion

8.4.1 Solid culture media

Two methods can be used: pour plate, or surface spread.

For pour plate method, the inoculated volume will be 1 ml of a 10^2 CFU/ml suspension of tested microorganisms.

For surface spread method, the inoculated volume will be 100 µl of a 10^3 CFU/ml suspension of tested microorganisms.

NOTE Filtration method can be applied.

For each tested microorganism, aseptically transfer in duplicate the relevant volume (1 ml or 100 µl) of the calibrated suspension prepared according to the used method, pour plate or surface spread, to deliver not more than 100 CFU in each fraction of medium.

Incubate for the appropriate time and temperature described in Annex A.

8.4.2 Liquid culture media

For each tested microorganism, aseptically transfer the relevant volume of the calibrated suspension to obtain not more than 100 CFU in each fraction of medium in tubes or bottles.

Incubate for the appropriate time and temperature described in Annex A and examine the media for macroscopic evidence of growth.

For coloured and/or turbid liquid culture media, subculture into or on solid culture media according to the used method, pour plate or surface spread.

8.5 Selective properties

8.5.1 Solid culture media — For indicative properties

For appropriate microorganism recommended in Annex A, aseptically transfer in duplicate the relevant volume of the calibrated suspension prepared in 8.2 to deliver not more than 100 CFU into each fraction of culture medium or on the surface of each fraction of culture medium using a spreader to disperse the inoculum and distribute it over the entire agar plate according to the used method pour plate or surface spread.

Incubate for the appropriate time and temperature in the conditions described in Annex A.

8.5.2 Solid culture media — For inhibition properties

For appropriate microorganism recommended in Annex A, aseptically transfer in duplicate the relevant volume of the calibrated suspension prepared in 8.2 to deliver at least 100 CFU into each fraction or on the surface of each fraction of culture medium using a spreader to disperse the inoculum and distribute it over the entire agar plate according to the used method pour plate or surface spread.

Incubate for the appropriate time and temperature in the conditions described in the Annex A.

9 Expression of results

9.1 Absence of microbial growth

9.1.1 Solid culture media

Note the presence or absence of colonies.

9.1.2 Liquid culture media

Note the visible growth or no growth in the liquid culture medium.

For coloured and/or turbid liquid culture media, note the presence or absence of colonies.

9.2 Growth promotion

9.2.1 Solid culture media

Average the number of colonies on the two plates from the new culture medium batch.

9.2.2 Liquid culture media

Note the visible growth or no growth in the liquid culture medium.

For coloured and/or turbid liquid culture media, note the presence or absence of colonies on the sub-cultured solid culture media.

9.3 Selective and indicative properties

9.3.1 Solid culture media — For indicative properties

Note the appearance and indication reaction of the colonies in the new culture medium batch.

9.3.2 Solid culture media — For inhibition properties

Note the presence or absence of colonies.

10 Interpretation and acceptance criteria

10.1 General

Recording all the results in a report is recommended. An example of culture media quality control report is provided in Annex B.

10.2 Absence of microbial growth

The portion of the culture medium incubated at the same temperature and duration as the tested microorganisms shall have no growth after incubation, to approve for the new culture medium batch.

10.3 Growth promotion

10.3.1 Solid culture media

To approve the new batch of culture medium the following acceptance criteria shall be met for each microorganism tested.

For detection tests of microorganisms, there shall be growth on the agar plates.

For enumeration tests of microorganisms, the average number of colonies on the plates from the new batch of culture medium shall not differ by a factor greater than 2 (i.e within 50 % to 200 %) of the average number of colonies from the initial microbial suspension. See Table 1.

NOTE The factor is introduced to take account of the variability of the method. It means that the result can be half or twice that of the inoculum. For example, with an inoculum of 100 CFU, acceptable counts are between: 100/2 = 50 CFU and 100 × 2 = 200 CFU.

Table 1 — Example of culture media quality control report

GROWTH PROMOTION	
Microorganisms & test conditions	
Strain:	*Staphylococcus aureus*
Incubation time:	48 h
Temperature:	(32,5 ± 2,5) °C
Reference medium:	Tryptic soy agar (TSA)
Inoculum:	100
Expected results:	50<Number of CFU <200
Number of CFU results:	74
Conform:	YES

10.3.2 Growth promotion — Liquid media culture

To approve the new batch of culture medium, visible growth shall be observed for each microorganism tested.

For coloured and/or turbid liquid culture media, there shall be growth on the sub-cultured agar plates.

10.4 Selective properties of solid culture media

10.4.1 For indicative properties

To approve the new batch of culture medium the following acceptance criteria shall be met for each microorganism tested.

— There shall be growth on the selective agar plates.

— To demonstrate the indicative performance of a culture medium, visually compare the colonies on plate to those enumerated in the initial suspension according to Annex A. The colonies should be similar in appearance.

10.4.2 For inhibition properties

No growth of the non-target test microorganism occurs.

Annex A
(normative)

Specifications for growth promoting, inhibitory and indicative properties of culture media

Table A.1 — Enumeration culture media

Culture medium	International Standard	Microorganisms	Properties	Incubation temperature	Incubation time[a]
Eugon LT agar medium	ISO 21149	*Staphylococcus aureus* *Escherichia coli* *Pseudomonas aeruginosa*	Growth promotion	(32,5 ± 2,5) °C	≤48 h
Potato dextrose agar (PDA)	ISO 11930	*Aspergillus brasiliensis*	Growth promotion	(22,5 ± 2,5) °C	≤3 d
Sabouraud dextrose agar medium (SDA)	ISO 11930	*Candida albicans*	Growth promotion	(32,5 ± 2,5) °C	≤48 h
Sabouraud dextrose agar medium (SDA)	ISO 16212	*Candida albicans*	Growth promotion	(22,5 ± 2,5) °C	≤5 d
	ISO 21322	*Candida albicans*	Growth promotion	(22,5 ± 2,5) °C	≤5 d
Sabouraud dextrose agar medium with chloramphenicol (SDCA)	ISO16212	*Candida albicans*	Growth promotion	(25 ± 2,5) °C	≤3 d
	ISO 21322	*Candida albicans*	Growth promotion	(25 ± 2,5) °C	≤3 d
Tryptic soy agar (TSA) or soybean casein digest agar medium (SCDA)	ISO 21149	*Staphylococcus aureus* *Escherichia coli* *Pseudomonas aeruginosa* *Candida albicans*	Growth promotion	(32,5 ± 2,5) °C	≤66 h

[a] For growth promotion test, the incubation time at the specified temperature is the shortest period of time specified in the relevant International Standard on cosmetics.

Table A.2 — Enrichment liquid culture media

Culture medium	International Standard	Microorganisms	Properties	Incubation temperature	Incubation time[a]
Eugon LT100, modified Eugon LT and other enrichment broths	ISO 18415	*Staphylococcus aureus* *Escherichia coli* *Pseudomonas aeruginosa* *Candida albicans*	Growth promotion	(32,5 ± 2,5) °C	≤20 h

[a] For growth promotion test, the incubation time at the specified temperature is the shortest period of time specified in the relevant International Standard on cosmetics.