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Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media

Microbiologie des aliments, des aliments pour animaux et de l'eau — Préparation, production, stockage et essais de performance des milieux de culture

Reference number
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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

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Foreword

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The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in collaboration with Technical Committee ISO/TC 147 *Water quality*, Subcommittee SC 4, *Microbiological methods*.

This first edition of ISO 11133 replaces the second edition of ISO/TS 11133-1 (ISO/TS 11133-1:2009) and the first edition of ISO/TS 11133-2:2003, which have been technically revised. It also incorporates the Amendment ISO/TS 11133-2:2003/Amd.1:2011. In particular, it also includes requirements for microbiology media for water testing. It supersedes ISO 9998:1991.

This corrected version of ISO 11133:2014 incorporates the following corrections:

— In Annex E

  Selective media for enumeration of microorganisms

  — DG18 column Incubation: d was replaced with days;

  — EC column control strain: £ was deleted after Pseudomonas;

  — mCCDA: [d] was deleted after both species of *Campylobacter*; [b] was added after 000156;

  — mCCDA: the criteria "Total or partial inhibition (0-1)" was added to the control stain *E. coli* and "Total inhibition (0)" was added to *S. aureus*;

  — TSC: the row with *Pseudomonas aeruginosa* and the WDCM number 00025 was deleted.

  Selective enrichment media

  — Bolton productivity: the cocktails of control strains were split into 2 separate cells;

  — EE: [d] was added before [i], after both stains of *Salmonella;*

  — ITC: a new cocktail of strains was introduced for Productivity;

  — PBS selectivity: [b] was added after 00025;

— RVS Productivity: added [d] to *E. coli*.

Non-selective liquid media

— mCCDA: [d] was deleted after both species of *Campylobacter*; [b] was added after 000156;

— mCCDA column Characteristic reactions: “colonies” was added after “moist”;

— PEMBA lane productivity: [i] was deleted after “good growth (2)”;

— Media TCBS was added after TBX;

— VRBG: one *Salmonella* Typhimurium was replaced by *Salmonella* Enteritidis WDCM 00030 and [d,i] was added to both *Salmonella*;

Non-selective isolation media

— Nutrient agar: the WDCM numbers were inverted between *S.* Typhimurium and *S.* Enteritidis;

— TSYEA: name and WDCM were corrected to *Listeria monocytogenes* 4b WDCM 00021[b];

Multipurpose media

— Pre-enrichment for Enterobacteriaceae: added [d] to both *Salmonella* and deleted “or” between the 2 WDCM numbers.

Reference media for enumeration of microorganisms

— TSA: deleted “*Escherichia coli* O157:H7 WDCM 00014 (non-toxigenic)”;

— SDA: added WDCM number 00053[b] to *Aspergillus*;

— In Annex F

Selective media for enumeration of microorganisms by comparing with a non-selective reference medium

— Colilert was replaced by Colilert-18 and the WDCM number 00207 was replaced by 00024.

Selective media for enumeration of microorganisms by comparing with a previously accepted batch (for use in special cases)

— Colilert was replaced by Colilert-18 and the WDCM number 00207 was replaced by 00024;

— Lactose TTC: a line was added between *Enterococcus faecalis* and *Pseudomonas aeruginosa* and the WDCM number corresponding.

Selective enrichment media

— Bolton/Preston Productivity: cocktails of control strains were split in 2 separate cells;

Non-selective liquid media

— “Saline salt” was replaced by “Saline solution”, and a [b] was added after 00034;

— mCCDA: [d] was deleted after both species of *Campylobacter*; [b] was added after 000156.

Introduction

In laboratories carrying out microbiological examinations, the main objectives are to maintain, resuscitate, grow, detect and/or enumerate a wide variety of microorganisms. Culture media are used in all traditional microbiological culture techniques and also for many alternative techniques. Many formulae of culture media are commercially available and many more, designed for specific growth purposes, are described in the literature.

Many tests and procedures depend upon culture media being capable of providing consistent and reproducible results. The requirements for media may be specific to both the sample and the organisms to be detected. Culture media meeting established performance criteria are therefore a pre-requisite for any reliable microbiological work. Sufficient testing should be carried out to demonstrate

a) the acceptability of each batch of medium,

b) that the medium is “fit for purpose”, and

c) that the medium can produce consistent results.

These three criteria are an essential part of internal quality control procedures and, with appropriate documentation, will permit effective monitoring of culture media and contribute to the production of both accurate and reliable data. For reliable microbiological analysis it is essential to use culture media of proven quality. For all media described in standard methods it is essential to define the minimum acceptance criteria required to ensure their reliability. It is recommended that in the determination of the performance characteristics of a culture medium tests are carried out that conform with this International Standard.

The establishment of widely accepted minimum performance criteria for media should lead to products with more consistent quality and thus reduce the extent of testing necessary in the user’s laboratory.

In addition the acceptance criteria measured by the methods defined in this International Standard can be used by all microbiological laboratories to evaluate the productive, selective and/or elective properties of a culture medium.

In the microbiological analysis of food, animal feed and water, the requirements of this International Standard have precedence in the assessment of culture media quality.

Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media

1 Scope

This International Standard defines terms related to quality assurance of culture media and specifies the requirements for the preparation of culture media intended for the microbiological analysis of food, animal feed, and samples from the food or feed production environment as well as all kinds of water intended for consumption or used in food production.

These requirements are applicable to all categories of culture media prepared for use in laboratories performing microbiological analyses.

This International Standard also sets criteria and describes methods for the performance testing of culture media. This International Standard applies to producers such as:

— commercial bodies producing and/or distributing ready-to-use or semi-finished reconstituted or dehydrated media;

— non-commercial bodies supplying media to third parties;

— microbiological laboratories preparing culture media for their own use.

2 Normative references

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 6887-1, *Microbiology of food and animal feed — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 6887-2, *Microbiology of food and animal feed — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products*

ISO 6887-3, *Microbiology of food and animal feed — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products*

ISO 6887-4, *Microbiology of food and animal feed — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 4: Specific rules for the preparation of miscellaneous products*

ISO 6887-5, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 5: Specific rules for the preparation of milk and milk products*

ISO 6887-6, *Microbiology of food and animal feed — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 6: Specific rules for the preparation of samples taken at the primary production stage*

ISO 7704, *Water quality — Evaluation of membrane filters used for microbiological analyses*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

ISO 8199, *Water quality — General guidance on the enumeration of micro-organisms by culture*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

NOTE 1 This clause gives the general definitions relating to quality assurance of culture media and provides terminology relating to performance testing, culture media and test microorganisms.

NOTE 2 Tables E.2 and F.2 give explanations of media name abbreviated terms.

3.1 General terms and definitions

3.1.1
quality control
part of quality management focused on fulfilling quality requirements

Note 1 to entry: See Reference [1].

3.1.2
batch of culture medium
lot of culture medium
homogeneous and fully traceable unit of a medium referring to a defined amount of bulk, semi-finished product or end product, which is consistent in type and quality and which has been produced within one defined production period, having been assigned the same batch (or lot) number

3.1.3
chromogenic substrate
fluorogenic substrate
substrate containing a chromophore/fluorophore group and a substrate utilizable by bacteria or fungi

Note 1 to entry: After splitting the chromogenic/fluorogenic substrate, the chromophore/fluorophore is released and a coloured/fluorescent end product becomes visible/can be detected using an ultraviolet (UV) lamp.

3.2 Terminology of performance testing

3.2.1
performance of culture medium
response of a culture medium to challenge by test organisms under defined conditions

3.2.2
target microorganism
microorganism or group of microorganisms to be detected or enumerated

3.2.3
non-target microorganism
microorganism that is suppressed by the medium and/or conditions of incubation or does not show expected characteristics of the target microorganism

3.2.4
productivity of culture medium
level of recovery of a target microorganism from the culture medium under defined conditions

3.2.5
selectivity of culture medium
degree of inhibition of a non-target microorganism on or in a selective culture medium under defined conditions

3.2.6
electivity of culture medium
specificity of culture medium
demonstration, under defined conditions, that non-target microorganisms do not show the same visual characteristics as target microorganisms

3.3 Terminology of culture media

3.3.1
culture medium
formulation of substances, in liquid, semi-solid or solid form, which contain natural and/or synthetic constituents intended to support the multiplication, (with or without inhibition of certain microorganisms), identification or preservation of viability of microorganisms

Note 1 to entry: When used in connection with compound words, this term is often shortened to read "medium" (e.g. enrichment medium).

3.3.2 Culture media classified by composition

3.3.2.1
chemically defined medium
culture medium consisting only of chemically defined constituents of known molecular structure and degree of purity

3.3.2.2
chemically undefined or partially undefined medium
culture medium consisting entirely or partly of natural materials, processed or otherwise, the chemical composition of which is not completely defined

Note 1 to entry: Harmonized designations for various chemically undefined components used in culture media are specified in Annex A.

3.3.2.3
chromogenic culture medium
fluorogenic culture medium
culture medium containing one or more chromogenic/fluorogenic substrates

Note 1 to entry: Chromogenic culture media facilitate the identification of bacteria or fungi by means of defined colour and morphological characteristics (culture medium typical growth). Fluorogenic media require visualization using a UV lamp. The biochemical reaction products, which are necessary for the efficiency of chromogenic/fluorogenic culture media, are normally the result of the enzymatic activity of certain organisms, which in turn depends greatly on the precise maintenance of specific conditions (e.g. temperature, pH value, concentrations of substrate).

3.3.3 Culture media classified by physical consistency

3.3.3.1
liquid medium
culture medium consisting of an aqueous solution of one or more constituents, such as peptone water and nutrient broth

Note 1 to entry: In some cases, solid particles are added to the liquid culture medium, such as cooked meat medium.

Note 2 to entry: Liquid media in tubes, flasks or bottles are commonly called "broths".

3.3.3.2
solid medium
semi-solid medium
liquid medium containing solidifying substances (e.g. agar-agar, gelatin) in different concentrations

Note 1 to entry: Due to the worldwide use of media solidified with agar-agar, the shortened term "agar" is often used synonymously for solid media and therefore in connection with nouns, e.g. "Plate Count agar".

Note 2 to entry: Solid media poured into Petri dishes are commonly called “plates”. Solid media poured into tubes or small bottles that are kept in slanted positions while the media are solidifying are often called “slants” or “slopes”. If the medium is dispensed to fill the bottom of the container, this forms a “butt”.

3.3.4 Culture media classified according to their use

3.3.4.1
transport medium
medium designed to preserve and maintain the viability of microorganisms whilst minimising numerical change in the time period between sample collection and laboratory processing of the sample

EXAMPLE Stuart or Amies transport medium

3.3.4.2
preservation medium
medium designed to preserve and maintain the viability of microorganisms over an extended period, to protect them against the adverse influences which may occur during long-term storage and to allow recovery after this period

EXAMPLE Dorset egg medium, nutrient agar slopes

3.3.4.3
diluent medium
suspension medium
medium designed to separate microorganisms from a solid test product into a liquid phase and/or to reduce their concentration by dilution without multiplication or inhibition during the time of contact

EXAMPLE Peptone salt solution

3.3.4.4
resuscitation medium
medium enabling stressed and damaged microorganisms to repair and recover their capacity for normal growth without necessarily promoting their multiplication

EXAMPLE Buffered peptone water

Note 1 to entry: A resuscitation medium may also be used as a pre-enrichment medium, e.g. buffered peptone water.

3.3.4.5
pre-enrichment medium
enrichment medium
generally liquid medium which, due to its composition, provides particularly favourable conditions for multiplication of microorganisms

EXAMPLE Tryptone soya broth

3.3.4.5.1
selective enrichment medium
enrichment medium that allows the multiplication of specific microorganisms whilst partially or totally inhibiting the growth of other microorganisms

EXAMPLE Rappaport-Vassiliadis soya peptone medium(RVS)

3.3.4.5.2
non-selective enrichment medium
enrichment medium that allows the growth of a wide variety of microorganisms

EXAMPLE Brain heart infusion broth

3.3.4.6
isolation medium
solid or semi-solid medium that allows the growth of microorganisms

3.3.4.6.1
selective isolation medium
isolation medium that allows growth of specific target microorganisms, while inhibiting, totally or partially, other microorganisms

EXAMPLE Modified charcoal cefoperazone deoxycholate agar (mCCD agar)

3.3.4.6.2
non-selective isolation medium
isolation medium that is not intended to selectively inhibit microorganisms

EXAMPLE Nutrient agar

3.3.4.6.3
chromogenic selective culture medium
fluorogenic selective culture medium
chromogenic/fluorogenic culture medium that also contains selective compounds which inhibit, totally or partially, accompanying flora occurring in test materials and thus support the precise detection of target microorganisms

EXAMPLE TBX agar, MUG/EC medium

3.3.4.7
differential medium
characterization medium
medium that permits the testing of one or more physiological/biochemical characteristics of the microorganisms for their identification

EXAMPLE TBX agar, Lactose agar with tergitol 7 and TTC

Note 1 to entry: Differential media that can be used as isolation media are referred to as isolation/differential media, e.g. Xylose lysine deoxycholate (XLD) agar, lactose TTC agar.

3.3.4.8
identification medium
medium designed to produce a specific identification reaction which usually does not require any additional confirmatory test

EXAMPLE Bile aesculin azide agar

3.3.4.9
enumeration medium
selective or non-selective culture medium that enables a quantification of the microorganisms

EXAMPLE Baird-Parker agar, Yeast extract agar

Note 1 to entry: An enumeration medium may include the properties of a resuscitation and/or enrichment medium.

3.3.4.10
confirmation medium
medium that contributes to the identification or characterization of the microorganism following a preliminary resuscitation and/or enrichment and/or isolation step

EXAMPLE Kligler iron agar

3.3.4.11
medium containing neutralisers
transport medium, dilution medium or culture medium containing neutralizing ingredients to inactivate detergents/disinfectants or other biocidal agents

3.3.4.12
medium having multiple uses
medium assigned to several categories

EXAMPLE Blood agar is a resuscitation medium according to 3.3.4.4, an isolation medium according to 3.4.4.6 and a differential medium according to 3.3.4.7 used for detection of haemolysis. Buffered peptone water is a diluent according to 3.3.4.3 and a pre-enrichment medium according to 3.3.4.5.

3.3.4.13
reference medium
medium, usually non-selective, for comparative evaluation of performance independent of the medium under test and demonstrated to be suitable for control use

EXAMPLE Tryptone soya agar (TSA)

3.3.5 Culture media classified according to preparation method

3.3.5.1
ready-to-use medium
liquid, solid or semi-solid medium that is supplied in plates, bottles, tubes or other containers, in ready-to-use form or ready-to-use after remelting or ready-to-use after remelting and supplementing

3.3.5.1.1
finished culture medium
medium in a form that is ready for inoculation

3.3.5.1.2
ready-to-use medium after remelting
medium to be remelted, for instance for use in the pour-plate technique or to be poured into Petri dishes

3.3.5.1.3
ready-to-use medium after remelting and supplementing
medium to be remelted, supplemented and dispensed before use (incomplete ready-to-use medium)

EXAMPLE Tryptose sulphite cycloserine (TSC) agar, Baird- Parker or Rabbit Plasma Fibrinogen (RPF) agar

3.3.5.2
medium prepared from commercially dehydrated formulations
medium in dry form which requires rehydration and processing before use, resulting in one of two kinds of media:

— a complete medium;

— an incomplete medium to which supplements are added before use

EXAMPLE Powders, compacted granules, lyophilized products

3.3.5.3
medium prepared from individual components
medium produced by a microbiology laboratory entirely from its individual ingredients

3.4 Terminology for test microorganisms

3.4.1
test organism
microorganism generally used for performance testing of culture media

Note 1 to entry: Test organisms are further defined according to their source (see 3.4.2 to 3.4.7).

3.4.2
reference strain
microorganism obtained directly from a reference culture collection, i.e. a culture collection, which is a member of the World Federation of Culture Collections (WFCC) or the European Culture Collections' Organisation (ECCO), and defined to at least the genus and species level, catalogued and described according to its characteristics and preferably originating from food, animal feed, the food or feed production environment or water as applicable

3.4.3
reference stock
set of separate identical cultures obtained by a single subculture from the reference strain either in the laboratory or from a supplier

3.4.4
stock culture
primary subculture from a reference stock

3.4.5
working culture
subculture from a reference stock or stock culture or a reference material, certified or not

3.4.6
reference material
RM
material containing a quantity of revivable microorganisms, sufficiently homogenous and stable with respect to quantity of revivable microorganisms, which has been established to be fit for its intended use in a measurement process

Note 1 to entry: See Reference [3].

3.4.7
certified reference material
CRM
reference material characterized by a metrologically valid procedure for the quantity of revivable microorganisms, accompanied by a certificate that provides the value of the specified quantity of revivable microorganisms, its associated uncertainty and a statement of metrological traceability

Note 1 to entry: See Reference [3].

4 Quality assurance management

4.1 Documentation

4.1.1 Documentation from manufacturer or producer

The following information shall be available from the manufacturer or producer (commercial or non-commercial bodies supplying media to third parties):

— name of the medium, individual components and any supplements and, if possible, their product codes;

— technical data sheet, e.g. formulation, intended use, filling quantity if applicable, references;

— safety and/or hazard data where needed;

— batch number;

— target pH of the complete medium;

— storage information and expiry date;