
**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*





COPYRIGHT PROTECTED DOCUMENT

© ISO 2014

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

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	v
Introduction	vi
1 Scope	1
2 Normative references	1
3 Terms and definitions	2
3.1 General terms and definitions	2
3.2 Terminology of performance testing	2
3.3 Terminology of culture media	3
3.4 Terminology for test microorganisms	6
4 Quality assurance management	7
4.1 Documentation	7
4.2 Storage	8
4.3 Laboratory preparation of media	8
4.4 Storage and shelf-life of prepared media	11
4.5 Preparation for use	12
4.6 Incubation of solid media in Petri dishes	14
4.7 Disposal of media	14
5 Test organisms for performance testing	14
5.1 General	14
5.2 Selection of test organisms	14
5.3 Preservation and maintenance of test organisms	15
5.4 Microorganisms for performance testing	16
6 Quality control and performance testing of culture media	19
6.1 General requirements	19
6.2 Physical and chemical quality control	19
6.3 Microbiological quality control	19
6.4 General requirements for microbiological performance testing	20
6.5 Performance evaluation and interpretation of results	21
6.6 Confirmation media and reagents	22
7 Methods for performance testing of solid culture media	22
7.1 General	22
7.2 Methods for quantitative tests	22
7.3 Testing of culture media used for membrane filtration	24
7.4 Methods for qualitative tests	24
8 Methods for performance testing of liquid culture media	25
8.1 General	25
8.2 Quantitative tube method for performance testing of liquid enrichment media (dilution to extinction method)	25
8.3 Qualitative tube method for performance testing of selective liquid media	26
8.4 Qualitative single tube method (turbidity) for performance testing of liquid media	27
9 Methods for performance testing of diluents and transport media	28
9.1 General	28
9.2 Method for testing diluents	28
9.3 Method for testing transport media	29
10 Documentation of test results	30
10.1 Information provided by the manufacturer	30
10.2 Traceability	30
Annex A (informative) Designation of the components of culture media in International Standards on microbiological analysis of food, animal feed and water	31

Annex B (normative) Preparation of reference stock and working culture	33
Annex C (normative) Flow charts of methods for performance testing	38
Annex D (informative) Example of card for recording test results of culture media	42
Annex E (normative) Test microorganisms and performance criteria for culture media commonly used in food microbiology	44
Annex F (normative) Test microorganisms and performance criteria for culture media commonly used in water microbiology	62
Annex G (normative) Use of control charts to monitor quantitative testing of solid culture media	73
Annex H (informative) Quality assurance of culture media — Troubleshooting	80
Annex I (informative) Quantitative testing of liquid media	82
Annex J (normative) Definition of microbiological performance tests for standardized culture media	86
Bibliography	90

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. www.iso.org/directives

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received. www.iso.org/patents

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 34, *Food products*, Subcommittee SC 9, *Food products*, 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.

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**selectivity 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