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

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

[Revision of second edition (ISO 11133-1:2009), first edition of ISO 11133-2:2003 and ISO 11133-2:2003/Amd.1:2011]

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Should this draft be accepted, a final draft, established on the basis of comments received, will be submitted to a parallel two-month approval vote in ISO and formal vote in CEN.

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 11133 was prepared by Technical Committee 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*, and by Technical Committee CEN/TC 275, *Food analysis - Horizontal methods*.

Annexes A, D, H and I are informative. Annexes B, C, E, F, G and J are normative.

This document combines the previously published two parts of ISO/TS 11133 and also includes requirements for microbiology media for water testing.

This document includes a Bibliography.

## 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

- I) the acceptability of each batch of medium ;
- II) that the medium is 'fit for purpose' and
- III) 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 assure 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.

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

## 1 Scope

This International Standard provides the general terminology related to quality assurance and specifies the requirements for the preparation of culture media to be used for the microbiological analysis of products intended for human consumption or animal feeding and samples from the food production environment as well as all kinds of water.

These requirements are applicable to four categories of culture media used in laboratories that prepare and/or use culture media for performing microbiological analyses:

- commercially manufactured ready-to-use media ;
- commercially manufactured media to be remelted, supplemented and distributed;
- media prepared from commercially available dehydrated formulations ;
- media prepared from their individual components.

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

- 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 feeding stuffs — 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 feeding stuffs — 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 feeding stuffs — 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 feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 4: Specific rules for the preparation of products other than milk and milk products, meat and meat products, and fish and fishery 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 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*

The following references are necessary to cover the range of microbiological methods described in this standard. Only the standards relevant to the laboratory are required.

ISO 4831, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique*

ISO 4832, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique*

ISO 4833, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of micro-organisms — Colony-count technique at 30 °C*

ISO 6222, *Water quality — Enumeration of culturable micro-organisms — Colony count by inoculation in a nutrient agar medium*

ISO 6461-1, *Water quality — Detection and enumeration of the spores of sulphite-reducing anaerobes (clostridia) — Part 1: Method by enrichment in a liquid medium*

ISO 6461-2, *Water quality — Detection and enumeration of the spores of sulphite-reducing anaerobes (clostridia) — Part 2: Method by membrane filtration*

ISO 6579, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Salmonella spp.*

ISO 6579 Amd 1, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Salmonella spp. — Amendment 1: Annex D: detection of Salmonella spp. in animal faeces and in environmental samples from the primary production stage*

ISO 6888-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 1: Technique using Baird-Parker agar medium*

ISO 6888-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 2: Technique using rabbit plasma fibrinogen agar medium*

ISO 6888-3, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 3: Detection and MPN technique for low numbers*

ISO 7251, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli — Most probable number technique*

ISO 7899-1, *Water quality — Detection and enumeration of intestinal enterococci — Part 1: Miniaturized method (Most Probable Number) for surface and waste water*

ISO 7899-2, *Water quality — Detection and enumeration of intestinal enterococci — Part 2: Membrane filtration method*

ISO 7932, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of presumptive Bacillus cereus — Colony-count technique at 30 °C*

ISO 7937, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of Clostridium perfringens — Colony-count technique*

ISO 9308-1, *Water quality — Detection and enumeration of Escherichia coli and coliform bacteria — Part 1: Membrane filtration method*

ISO 9308-2, *Water quality — Detection and enumeration of Escherichia coli and coliform bacteria — Part 2: Most probable number method*

ISO 9308-3, *Water quality — Detection and enumeration of Escherichia coli and coliform bacteria — Part 3: Miniaturized method (Most Probable Number) for the detection and enumeration of E.coli in surface and waste water*

ISO 10272-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of Campylobacter spp. — Part 1: Detection method*

ISO/TS 10272-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of Campylobacter spp. — Part 2: Colony count technique*

ISO 10272-3, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Campylobacter spp. — Part 3: Semi-quantitative method*

ISO 10273, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica*

ISO/TS 11059 IDF/RM 225, *Milk and milk products — Method for the enumeration of Pseudomonas spp.*

ISO 11290-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of Listeria monocytogenes — Part 1 : Detection method*

ISO 11290-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of Listeria monocytogenes — Part 2 : Enumeration method*

ISO 11731-1, *Water quality — Detection and enumeration of Legionella — Part 1: General method*

ISO 11731-2, *Water quality — Detection and enumeration of Legionella — Part 2: Direct membrane filtration method for waters with low bacterial counts*

ISO 13720, *Meat and Meat products — Enumeration of presumptive Pseudomonas spp.*

ISO 15213, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions*

ISO 15214, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of mesophilic lactic acid bacteria. Colony-count technique at 30° C*

ISO 16266, *Water quality — Detection and enumeration of Pseudomonas aeruginosa — Method by membrane filtration*

ISO 16649-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli — Part 1: Colony-count technique at 44 degrees celsius using membranes and 5-bromo-4-chloro-3-indolyl beta-D-glucuronide*

ISO 16649-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli — Part 2: Colony-count technique at 44 degrees celsius using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide*

ISO 16649-3, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli — Part 3: Most probable number technique using 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide*

ISO 16654, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Escherichia coli O157*

ISO 17995, *Water quality — Detection and enumeration of thermotolerant Campylobacter species*

ISO 19250, *Water quality — Detection of Salmonella spp.*

ISO 21527-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds — Part 1: Colony count technique in products with water activity greater than 0.95*

ISO 21527-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds — Part 2: Colony count technique in products with water activity less than or equal to 0.95*

ISO 21528-1, *Microbiology of food and animal feeding stuffs — Horizontal methods for the detection and enumeration of Enterobacteriaceae — Part 1: Detection and enumeration by MPN technique with pre-enrichment*

ISO 21528-2, *Microbiology of food and animal feeding stuffs — Horizontal methods for the detection and enumeration of Enterobacteriaceae — Part 2: Colony-count method*

ISO 21871, *Microbiology of food and animal feeding stuffs - Horizontal methods for the determination of low numbers of presumptive Bacillus cereus – Most probable number technique and detection method*

ISO 21872-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of potentially pathogenic Vibrio spp. — Part 1: Detection of Vibrio parahaemolyticus and Vibrio cholerae*

ISO 21872-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of potentially pathogenic Vibrio spp. — Part 2: Detection of species other than Vibrio parahaemolyticus and Vibrio cholerae*

Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption Off J Eur Comm 5.12.98 L 330/32

### **3 General terms and definitions**

#### **3.1 Introduction**

This section gives the general definitions relating to quality assurance and provides terminology relating to culture media and to control cultures.

#### **3.2 General terminology**

##### **3.2.1**

##### **quality control**

technical operations and activities that are used to fulfil the requirements for quality

**3.2.2****batch 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 passed the requirements of production (in-process control) and performance testing, and which has been produced within one defined production period, having been assigned the same number

**3.2.3****chromogenic substrate**

substrate containing a chromophore group and a substrate utilizable by bacteria or fungi; after splitting the chromogenic substrate, the chromophore is released and a coloured end product becomes visible

**3.3 Terminology of performance testing****3.3.1****performance of culture medium**

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

**3.3.2****productivity of culture medium**

level of recovery of a target organism from the culture medium under defined conditions

**3.3.3****selectivity of culture medium**

degree of inhibition of a non-target (unwanted) organism on or in a selective culture medium under defined conditions

**3.3.4****specificity of culture medium**

demonstration of specified visual characteristics by target microorganisms but not by non-target (unwanted) microorganisms under defined conditions

**3.4 Terminology of culture media****3.4.1****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 When used in connection with compound words, this term is often shortened into "medium" (e.g. enrichment medium).

**3.4.2 Culture media classified by composition****3.4.2.1****chemically defined medium**

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

**3.4.2.2****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 Harmonised designations for various chemically undefined components used in culture media are specified in Annex A