

Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media —

Part 2: Practical guidelines on performance testing of culture media

ICS 07.100.30

National foreword

This Draft for Development is the official English language version of CEN ISO/TS 11133-2:2003+A1:2011. It supersedes DD CEN ISO/TS 11133-2:2003, which is withdrawn.

The start and finish of text introduced or altered by amendment is indicated in the text by tags. Tags indicating changes to ISO text carry the number of the ISO amendment. For example, text altered by ISO amendment 1 is indicated by A1 A1.

This publication is not to be regarded as a British Standard.

It is being issued in the Draft for Development series of publications and is of a provisional nature because the establishment of minimum performance criteria for media that are widely accepted should lead to more consistent commercially made products that in consequence would reduce the extent of testing in the users laboratory. It should be applied on this provisional basis, so that information and experience of its practical application may be obtained.

Comments arising from the use of this Draft for Development are requested so that UK experience can be reported to the European organization responsible for its conversion to a European standard. A review of this publication will be initiated 2 years after its publication by the European organization so that a decision can be taken on its status at the end of its 3-year life. Notification of the start of the review period will be made in an announcement in the appropriate issue of *Update Standards*.

According to the replies received by the end of the review period, the responsible BSI Committee will decide whether to support the conversion into a European Standard, to extend the life of the Technical Specification or to withdraw it. Comments should be sent in writing to the Secretary of BSI Technical Committee AW/9, Microbiology, at 389 Chiswick High Road, London W4 4AL, giving the document reference and clause number and proposing, where possible, an appropriate revision of the text.

A list of organizations represented on this committee can be obtained on request to its secretary.

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English version

Microbiology of food and animal feeding stuffs - Guidelines on
preparation and production of culture media - Part 2: Practical
guidelines on performance testing of culture media (ISO/TS
11133-2:2003)

Microbiologie des aliments - Guide pour la préparation et la
production des milieux de culture - Partie 2: Guide général
pour les essais de performance des milieux de culture
(ISO/TS 11133-2:2003)

Mikrobiologie von Lebensmitteln und Futtermitteln -
Anleitung für die Vorbereitung und Herstellung von
Nährmedien - Teil 2: Praktische Anleitung zur
Leistungsprüfung von Nährmedien (ISO/TS 11133-2:2003)

This Technical Specification (CEN ISO/TS) was approved by CEN on 8 December 2002 for provisional application.

The period of validity of this CEN ISO/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN ISO/TS can be converted into a European Standard.

CEN members are required to announce the existence of this CEN ISO/TS in the same way as for an EN and to make the CEN ISO/TS available. It is permissible to keep conflicting national standards in force (in parallel to the CEN ISO/TS) until the final decision about the possible conversion of the CEN ISO/TS into an EN is reached.

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Contents

Page

Foreword	3
Introduction	4
1 Scope	4
2 Normative references	4
3 Terms and definitions	6
4 Criteria for routine quality control	6
5 Methods for use in performance testing of culture media	9
6 Documentation of test results	15
Annex A (informative) Example of card for recording test results of culture media prepared by the user laboratory	17
Annex B (normative) Recommended test microorganisms for commonly used culture media (giving information on the culture medium, culture conditions, test microorganisms, culture collection number of test organisms and the expected reactions)	18
Bibliography	27

Foreword

This document (CEN ISO/TS 11133-2:2003) has been prepared by Technical Committee CEN/TC 275 "Food analysis - Horizontal methods", the secretariat of which is held by DIN, in collaboration with Technical Committee ISO/TC 34 "Food products".

This document "Microbiology of food and animal feeding stuffs – Guidelines on preparation and production of culture media" consist of two parts:

¾ Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory

¾ Part 2: Practical guidelines on performance testing of culture media

Annex A is informative. Annex B is normative.

This document includes a Bibliography.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to announce this Technical Specification: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Slovakia, Spain, Sweden, Switzerland and the United Kingdom.

Foreword to amendment 1

This document (CEN ISO/TS 11133-2:2003/A1:2011) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 275 "Food analysis - Horizontal methods" the secretariat of which is held by DIN.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

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Endorsement notice

The text of ISO/TS 11133-2:2003/AMD 1:2011 has been approved by CEN as a CEN ISO/TS 11133-2:2003/A1:2011 without any modification.

Introduction

It is essential to use culture media of proven quality to carry out microbiological analysis of food reliably. For all media described in standardized methods it is essential to define the minimum acceptance criteria required to ensure media reliability. It is recommended that in the determination of the performance characteristics of a culture medium tests are carried out that conform with this Technical Specification. This applies to:

- a) commercially prepared ready-to-use or dehydrated media;
- b) culture media prepared from basic constituents in the user's laboratory.

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

Furthermore the minimum acceptance criteria measured by the methods defined in this Technical Specification 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 and animal feeding stuffs the requirements of this Technical Specification have priority in the assessment of media quality.

1 Scope

This Technical Specification sets criteria and methods for the performance testing of culture media. This Technical Specification applies to:

- ¾ commercial bodies producing and/or distributing ready-to-use or semi-finished reconstituted or dehydrated media to microbiological laboratories;
- ¾ non-commercial bodies supplying media to third parties;
- ¾ microbiological laboratories preparing culture media for their own use and evaluating the performance of these media.

2 Normative references

This Technical Specification incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this Technical Specification only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

ENV ISO 11133-1:2000, *Microbiology of food and animal feeding stuffs – Guidelines on preparation and production of culture media – Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory (ISO/TR 11133-1:2000)*.

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* 

A1 ISO 6579, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Salmonella spp.*

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

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

ISO/TS 10272-3, *Microbiology of food and animal feeding stuffs — Horizontal method for detection and enumeration 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 11290-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of Listeria monocytogenes — Part 1: Detection method **A1***

A1 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 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 16649 (all parts), *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli*

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

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* **A1**

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ENV ISO 11133-1:2000 apply.

4 Criteria for routine quality control

4.1 General quality criteria

4.1.1 Quality of culture media

The quality of culture media depends on the quality of the basic ingredients, correct formulation, quality of preparation procedures, elimination of contaminant microbial agents and appropriate packaging and storage conditions (see ENV ISO 11133-1).

The manufacturer or producer in the laboratory shall comply with the physico-chemical characteristics of the culture media as specified in the corresponding standard. Furthermore, quality assessment shall ensure that the culture medium conforms to stated recommendations, including:

- $\frac{3}{4}$ distributed quantity and/or thickness;
- $\frac{3}{4}$ appearance, colour and homogeneity;
- $\frac{3}{4}$ gel consistency;
- $\frac{3}{4}$ moisture content;
- $\frac{3}{4}$ pH value;
- $\frac{3}{4}$ buffering capacity;
- $\frac{3}{4}$ microbial contamination.

The individual components and any nutritive or selective supplements shall also undergo suitable quality assessment procedures.

4.1.2 Quality of basic media components

Culture media described in the International Standards were judged satisfactory; however, due to the variability of their quality, it may be acceptable for media manufacturers to modify the concentration of some basic biological ingredients, as listed below:

- $\frac{3}{4}$ peptones and meat or yeast extracts variable in their nutritive properties;
- $\frac{3}{4}$ agar variable in its gelling properties;
- $\frac{3}{4}$ buffering substances;
- $\frac{3}{4}$ bile salts, bile extract and desoxycholate, antibacterial dyes, depending on their selective properties;
- $\frac{3}{4}$ antibiotics depending on their activity.

4.2 Microbiological quality criteria

4.2.1 General

The microbiological performance tests shall be carried out on a sample which is representative of a batch of end product.

4.2.2 Microbial contamination

An appropriate quantity, depending on the size of the batch of culture medium, shall be tested for microbial contamination by incubation under appropriate conditions. Target limits for the percentage of contaminated plates or containers of liquid medium should be established for each medium or specified by the manufacturer. Manufacturers should draw up specifications based on media components, processing limits and type of packaging.

NOTE 1 The samples to be tested should be at least 1 plate or tube or 1 % of plates or tubes from the beginning and 1 plate or tube or 1 % of plates or tubes from the end of a pouring or dispensing process. The plates or tubes should be incubated for at least 18 h at 37°C or under the incubation conditions which are used routinely for this medium according to the specific standard.

NOTE 2 For statistical sampling plans refer to the ISO 2859-1:1999.

4.2.3 Growth

4.2.3.1 General

To evaluate each batch of complete culture medium, nutrient components or supplements, growth shall be appropriately assessed by either:

- a) quantitative; or
- b) semi-quantitative; or
- c) qualitative methods.

Quantitative, semi-quantitative or qualitative evaluations shall be performed by the methods described in this Technical Specification or by another generally accepted technique. For interpretation of the results of testing, it is necessary to compare the amount of growth on the test medium with that on a reference medium. The use of a specific reference medium is therefore mandatory for quantitative methods (see the specific standard or Annex B)

For semi-quantitative or qualitative methods, the use of a specific reference medium (see corresponding specific standard or Annex B) or a culture medium giving a "positive" reaction helps to interpret results. The reference medium must be of known good quality chosen from a recently released batch, or, if comparing long term stability, a batch from another supplier or a ready-to-use medium, etc.