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Mikrobiologija ġivil in krme - Navodila za pripravo in izdelavo gojiġļ - 2. del: Praktiļ na navodila za vrednotenje gojiġļ (ISO/TS 11133-2:2003)

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)

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)

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)

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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:

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

— *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.

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:

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

— non-commercial bodies supplying media to third parties;

— 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).*

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:

— distributed quantity and/or thickness;

— appearance, colour and homogeneity;

— gel consistency;

— moisture content;

— pH value;

— buffering capacity;

— 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:

— peptones and meat or yeast extracts variable in their nutritive properties;

— agar variable in its gelling properties;

— buffering substances;

— bile salts, bile extract and desoxycholate, antibacterial dyes, depending on their selective properties;

— 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 37ºC 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.

In addition, growth of the target strains shall be typical in appearance, size and morphology of the colonies and growth of the non-target strains shall be partly or completely inhibited.

4.2.3.2 Productivity

Solid, semi-solid or liquid culture media shall be inoculated with an appropriate inoculum (5.2.1.1) of the working culture of each of the defined test microorganisms using an appropriate device.

Productivity shall reach a defined minimum limit (see corresponding specific standard or Annex B).

For quantitative methods the Productivity Ratio P_R (1) is determined as follows:

$$P_R = \frac{N_S}{N_O} \quad (1)$$

where

N_s is the total colony count obtained on the culture medium under test (obtained from one or more plates);

N_0 is the total colony count obtained on the defined reference culture medium obtained from one or more plates, and shall be ≥ 100 cfu.

NOTE The Productivity Ratio of a non selective medium is at least 0,7 for microorganisms that can grow easily on that medium. The P_R of the target microorganisms on a selective medium should be at least 0,1. These values are generally achievable, however less rigorous criteria can be accepted for certain combinations of media and test microorganisms (see corresponding specific standard or Annex B)

For semi-quantitative methods, the scores of consecutive sectors of a plate inoculated by the ecometric technique are summed to obtain the growth index G_I, which varies according to the culture medium. It is therefore important to compare them with previous indices and/or G_I of a reference medium and to ensure that variations are not excessive. The expected range of variations for each culture medium can also be established once sufficient experience of the method has been gained.

Qualitative evaluations shall be carried out visually by allocating growth scores.

4.2.3.3 Selectivity

To assess selectivity quantitatively, selective culture media and a reference medium are inoculated with an appropriate inoculum (5.2.1.2.) of the defined test microorganism using an appropriate device. Selectivity has to reach defined values (see corresponding specific standard or Annex B).

The Selectivity Factor S_F (2), is calculated as follows:

$$S_F = D_O - D_S \quad (2)$$

where

D_O is the highest dilution showing growth of at least 10 colonies on the reference medium;

D_S is the highest dilution showing comparable growth on the test medium.

S_F, D_O and D_S are expressed in $\log_{10}$ units.

NOTE 1 If e.g. D_O 10^{-4} = $\log_{10}$ 4,0 and D_S 10^{-3} = $\log_{10}$ 3,0 then the selectivity factor is S_F = 1,0.

NOTE 2 The S_F of non-target microorganisms on a selective medium should be at least 2. This value is generally achievable. However, less rigorous criteria can be accepted for certain combinations of media and test microorganisms (see corresponding specific standard or Annex B).

For semi-quantitative and qualitative methods the growth of the non-target strain(s) shall be inhibited partly or completely.

4.2.4 Biochemical and physiological characteristics (selectivity and specificity)

The colony morphology and the diagnostic features together with the degree of selectivity should be established in order to obtain a complete picture of the performance of a medium.

The essential characteristics of specificity shall be defined and achieved. For differential media the quality of biochemical / physiological characteristics of the target microorganism(s) and the degree of inhibition of non-target microorganisms should be determined with an appropriate set of test strains.

4.2.5 Antimicrobial testing characteristics

The antimicrobial action of antibiotics depends upon their agar diffusion characteristics and any antagonistic effects from the components present. Media for testing the presence or absence of antimicrobial substances in food samples should conform to reference methods.

4.3 Performance evaluation and interpretation of results

A batch of culture medium performs satisfactorily if all the test microorganisms used perform according to the given specifications. It shall be accepted if both general and microbiological quality criteria are met.

5 Methods for use in performance testing of culture media

5.1 General

Examples of quantitative, semi-quantitative and qualitative testing methods for solid culture media and liquid media are described. In most cases in the user's laboratory semi-quantitative and qualitative techniques will meet the performance testing requirements of a batch of culture medium.

For special cases, e.g. evaluation of a new medium or a new manufacturer, etc., quantitative testing methods shall be performed by the user's laboratory.

Familiarity with general microbiological techniques is assumed and therefore the methods are not given in exhaustive detail.

Suitable test microorganisms are listed in Annex B (see also ENV ISO 11133-1).

NOTE It is the intention in the future, that new and revised individual standards for detection or enumeration of specific microorganisms or groups of microorganisms will describe the relevant test microorganisms to be used, together with the acceptance criteria for each culture medium in the standard.

In liquid media the interactions leading to the successful growth of microorganisms are more complex, hence defining performance testing methods is less straightforward than for solid media.

For the successful isolation of targeted microorganisms in a multistage method, for example detection of *Salmonella,* several complex interactions take place at each growth stage. Here a control test using appropriate samples, culture and reference materials should be set up, so that the productivity or the sensitivity, respectively, of the whole method is demonstrated. This is in addition to demonstrating that each component medium is fit for purpose.

5.2 Test microorganisms

The appropriate reference strains of target (productivity) and non-target (selectivity) microorganisms for each culture medium are given in Annex B. The test microorganisms should meet the requirements given in 5.2.2 of ENV ISO 11133-1:2000, e.g. robust, weakly growing, biochemically unreactive or injured strains, as appropriate.

Guidance on the preservation and maintenance of reference strains is given in Annex B of CEN/TS ISO/TC 11133-1.

5.2.1 Preparation of the working culture

Working cultures shall be prepared as a pure stationary phase culture in a non-selective broth from the reference stock culture.

Different techniques may be used, but shall guarantee the purity of the inoculum, as well as its standardisation which allows it to be used at a later stage.

NOTE Frozen inocula may be used if it can be shown that the microorganism can survive for the chosen period.

5.2.1.1 Working culture for productivity testing

For semi-quantitative or qualitative tests and productivity testing of target microorganisms an inoculum level is used to obtain 10 cfu to 100 cfu per plate or tube of medium.

5.2.1.2 Working culture for selectivity testing

For selectivity testing of culture media a suspension of the non-target microorganism containing 10^4 cfu to 10^6 cfu per ml is inoculated onto the plate or into the tube of medium.

5.2.1.3 Incubation conditions

Incubate the inoculated culture media in accordance with the conditions described in the corresponding standard and given in the appropriate tables in Annex B.

5.3 Methods for solid culture media

5.3.1 Quantitative plating method

5.3.1.1 General

This is a general method suitable for most solid culture media. It may not be suitable for testing some types of moulds.

5.3.1.2 Procedure

— Use working cultures as described in 5.2.1.

— Select an appropriate number of plates representative of each batch to be tested and ensure the surface of each plate is adequately dried. Plates of the reference medium should be similarly prepared (see 4.4.4 of ENV ISO 11133-1:2000).

— Spread onto the surface of the test and reference plates an inoculum of the diluted working culture to give counts that fall within the recommended limits given in 5.2.1.

NOTE 1 The modified Miles-Misra surface drop method and other dropping systems or a spiral plater may also be used.

NOTE 2 The pour plate method may also be used for culture media normally used for enumeration in this way. Incubate plates under appropriate conditions as defined in the individual standards.

— Count the colonies present on each plate or from each drop as appropriate. Assess the size and appearance of the colonies.

5.3.1.3 Calculation

Based on the volume spread on the plates and the dilution factor, the mean count on the medium can be calculated. In the case of dropping methods the number of drops and their volume must be considered.

5.3.1.4 Interpretation of results

To interpret the results, the Productivity Ratio P_R (4.2.3.2), and where appropriate the Selectivity Factor S_F (4.2.3.3), should be calculated.

5.3.2 Semi-quantitative streaking method based on ecometry

5.3.2.1 General

The streaking method is suitable for performance testing of solid and liquid culture media but the method is only semi-quantitative. Growth indices are therefore only indicative and it can only be regarded as a supplementary test for solid culture media.

When using this method the culture media tested should be dried to the same degree and the whole procedure shall be standardized so that results of different batches can be compared.

5.3.2.2 Procedure

— Agar plates are prepared in the usual manner with about 15 ml of agar. Media normally used for the pour plate technique, for example Plate Count Agar (PCA), may also be tested by surface plating on solidified media.

— Use working cultures as described in 5.2.1.

— The plates are streaked as shown in Figure 1 using a 1 µl loop. Four parallel lines are drawn with the loop at approximately 0,5 cm intervals over sector A. Streaking is repeated for sectors B and C and terminated in sector D with a single line. A template can be used beneath the plate to facilitate accurate streaking.

— The incubation times and temperatures stated in the standard methods are used.

NOTE Only the loop, not the wire, should be dipped in the culture. The loop should be completely filled with the culture. Excess liquid should be removed by pressing the wider part of the loop three times against the edge of the container. When streaking plates the angle between the loop and agar surface should be 20° to 30°. The pressure of the loop on the agar surface and the rapidity of streaking must be consistent throughout. Dipping the loop in the culture whilst foam and/or bubbles are on the surface of the broth should be avoided.

Normally the same loop is used for streaking all sectors from A to D without flaming the loop between streaks. In some cases where a lower growth index G_I is expected to demonstrate distinct differences, changing or sterilising the loop between streaking sectors A and B may be appropriate.