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**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**

*Microbiologie des aliments — Lignes directrices pour la préparation et  
la production des milieux de culture —*

*Partie 1: Lignes directrices générales d'assurance qualité pour la  
préparation des milieux de culture en laboratoire*



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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.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of document:

- an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;
- an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

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/TS 11133-1 was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 275, *Food analysis — Horizontal methods*, in collaboration with Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO/TS 11133-1:2000), which has been technically revised.

ISO/TS 11133 consists of the following parts, under the general title *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*
- *Part 2: Practical guidelines on performance testing of culture media*

## Introduction

Culture media are used in all traditional culture techniques and also for many alternative techniques. In the microbiology laboratory, many tests and procedures depend upon culture media being consistent and providing reproducible results. Many formulations of dehydrated culture media are commercially available and many more, designed for specific growth purposes, are described in the literature. In laboratories carrying out microbiological examinations of foods and feedstuffs, the main objectives are to maintain, resuscitate, grow, detect and/or enumerate a wide variety of microorganisms. The requirements for media are specific to both the sample and the organisms to be detected. Culture media meeting established or minimal performance criteria are therefore a prerequisite 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 precise data.

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# 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

### 1 Scope

This part of ISO/TS 11133 provides the general terminology related to quality assurance and specifies the minimum requirements for the preparation of culture media to be used for the microbiological analysis of products intended for human consumption or animal feeding.

It is also applicable to culture media to be used for the microbiological analysis of 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;
- media to be remelted, supplemented and distributed;
- media prepared from commercially available dehydrated formulations;
- media prepared from their individual components.

### 2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

ISO 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*

### 3 Terms and definitions

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

#### 3.1 General

##### 3.1.1

##### quality control

(food and feedstuffs) technical operations and activities that are used to fulfil the requirements for quality

**3.1.2**

**batch of culture media**

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

**3.1.3**

**performance of culture media**

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

**3.2 Culture media**

**3.2.1**

**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 to "medium" (e.g. enrichment medium).

**3.2.2**

**chemically defined medium**

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

**3.2.3**

**chemically undefined medium**

**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 Harmonized designations for various chemically undefined components used in culture media are specified in Annex A.

**3.2.4**

**liquid medium**

culture medium consisting of an aqueous solution of one or more constituents

EXAMPLES Peptone water, nutrient broth.

NOTE 1 In some cases, solid particles are added to the liquid culture medium.

NOTE 2 A liquid medium in tubes, flasks or bottles is commonly called "broth".

**3.2.5**

**solid medium**

**semi-solid medium**

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

NOTE 1 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 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".

**3.2.6**

**transport medium**

medium designed to preserve and maintain the viability of microorganisms without permitting significant multiplication in the time period between sample collection and laboratory processing of the sample

NOTE Transport media usually contain substances that do not permit multiplication of microorganisms but ensure their maintenance, e.g. Stuart's or Amies's transport medium.

**3.2.7****preservation medium**

medium designed to 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

EXAMPLES Dorset egg medium, nutrient agar slopes.

**3.2.8****suspension medium**

medium designed to separate microorganisms from a test product into a liquid phase without multiplication or inhibition during the time of contact

EXAMPLE Peptone saline solution.

NOTE 1 Suspension media are also used for dilution purposes.

NOTE 2 Suspension medium is commonly called "diluent".

**3.2.9****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 This may also be used as a pre-enrichment medium.

**3.2.10****pre-enrichment medium****enrichment medium**

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

**3.2.11****selective enrichment medium**

enrichment medium which allows the multiplication of specific microorganisms whilst partially or totally inhibiting the growth of other microorganisms

EXAMPLE Rappaport-Vassiliadis soya medium.

**3.2.12****non-selective enrichment medium**

enrichment medium which allows the growth of a wide variety of microorganisms

EXAMPLE Nutrient broth.

**3.2.13****isolation medium**

solid or semi-solid medium which allows the growth of microorganisms

EXAMPLE Plate count agar.

**3.2.14****selective isolation medium**

isolation medium which allows growth of specific target microorganisms, while inhibiting other microorganisms

EXAMPLE XLD agar.

**3.2.15**

**non-selective isolation medium**

isolation medium which is not intended to selectively inhibit microorganisms

EXAMPLE Plate count agar.

**3.2.16**

**differential medium  
characterization medium**

medium which permits the testing of one or more physiological/biochemical characteristics of the microorganisms for their identification

EXAMPLE MacConkey agar.

NOTE Differential media which can be used as isolation media are referred to as isolation/differential media, e.g. XLD agar, lactose TTC agar.

**3.2.17**

**identification medium**

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

EXAMPLE Bile esculin agar, TBX agar.

NOTE Identification media which can be used as isolation media are referred to as isolation/identification media.

**3.2.18**

**enumeration medium**

selective or non-selective culture medium which enables a quantification of the microorganisms

NOTE An enumeration medium can include the properties of a resuscitation and/or enrichment medium.

**3.2.19**

**confirmation medium**

medium which contributes partly or wholly to the identification or to the characterization of the microorganisms following a preliminary resuscitation, isolation and/or enrichment stage

EXAMPLE Kligler agar.

**3.2.20**

**medium having multiple uses**

medium assigned to several categories

EXAMPLE Blood agar is a resuscitation medium according to 3.2.9, an isolation medium according to 3.2.13 and a differential medium according to 3.2.16 used for detection of haemolysis.

**3.2.21**

**ready-to-use medium**

liquid, solid or semi-solid medium which is supplied in containers in ready-to-use form or ready-to-use after remelting

EXAMPLES Plates, tubes or other containers:

- complete ready-to-use medium;
- medium to be remelted, e.g. for use in pour-plate technique;
- medium to be remelted and dispensed before use, e.g. to be poured into Petri dishes;
- medium to be remelted, supplemented and dispensed before use, e.g. TSC medium, Baird Parker RPF agar.

**3.2.22****medium prepared from commercially dehydrated formulations**

medium in dry form which requires rehydration and processing prior to use

EXAMPLES Powders, granules, lyophilized products, resulting in one of two kinds of media:

- a complete medium;
- an incomplete medium to which supplements are added before use.

**3.2.23****medium prepared from individual components**

medium entirely produced from the complete formula of its specific ingredients

**3.3 Test microorganisms****3.3.1****test organisms**

microorganisms generally used for performance testing of culture media

NOTE Test organisms are further defined according to their source (see 3.3.2 to 3.3.5).

**3.3.2****reference strain**

microorganism obtained directly from an official culture collection and defined to at least the genus and species level, catalogued and described according to its characteristics and preferably originating from food or water as applicable

**3.3.3****reference stock**

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

**3.3.4****stock culture**

primary subculture from a reference stock

**3.3.5****working culture**

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

NOTE Reference material is a material containing a quantity of revivable microorganisms in a homogeneous, stable concentration. A certified reference material is a reference material for which the concentration is certified.

**4 Quality assurance of culture media****4.1 Documentation****4.1.1 Documentation from manufacturer or producer**

The following details should be available from the manufacturer or producer:

- name of the medium, individual components and any supplements and their product codes;
- batch number;
- target pH of the complete medium;

- storage information and expiry date;
- quality control certificate and test organisms used;
- results of performance testing with criteria of acceptance;
- technical data sheet;
- safety and/or hazard data where needed.

#### 4.1.2 Delivery acceptance of products

For each batch of product (ingredient or culture medium), check the following:

- identification of the product;
- integrity of packaging;
- expiry date of the product;
- documentation supplied.

Record the date of receipt.

## 4.2 Storage

### 4.2.1 General

In all cases, follow the manufacturer's instructions regarding storage conditions, expiry date and use.

### 4.2.2 Quality management and control for dehydrated media and supplements

Media are delivered as dehydrated powders or in granulated form in sealed containers. Supplements of different selective or diagnostic substances are supplied in either the lyophilized or liquid state. Purchases should be planned to encourage a regular turnover of stock (i.e. first in-first out). To maintain an inventory:

- check the seal;
- record date of first opening;
- assess visually the contents of opened containers.

Especially after opening a new container, the quality of the medium may depend on the storage environment. Loss of quality of dehydrated media is shown by change in flow characteristics of the powder, homogeneity, caking, colour changes, etc. Any dehydrated medium that has absorbed moisture or shows obvious changes in physical appearance shall be discarded.

### 4.2.3 Commercially supplied ready-to-use media

Follow the manufacturer's instructions regarding storage conditions, expiry date and use.

### 4.2.4 Laboratory prepared media

The shelf-life of media varies. Specific International Standards or national standards may stipulate specific conditions and shelf-life.

Store the media under conditions which prevent any modification of their composition, namely protected from light and desiccation and in a refrigerator at  $5\text{ °C} \pm 3\text{ °C}$ , if necessary. It is generally recommended not to exceed 2 to 4 weeks of storage for plates and 3 to 6 months for bottles and tubes, unless otherwise specified in specific standards or results of the laboratory shelf-life validation indicate a longer shelf-life.

It is recommended that media to which labile supplements have been added should be used on the day of preparation, unless otherwise specified in specific standards or results of the laboratory shelf-life validation indicate a longer shelf-life. Solid media containing chemically reactive and/or labile substances should not be stored in bulk for remelting.

A validated expiry date for stored media should be established. Observe any colour change, sign of evaporation/dehydration or microbial growth. Batches of media showing such changes shall not be used.

Prior to use or before further heating, it is recommended that the culture media are equilibrated to ambient temperature.

NOTE Special instructions for storage of media as plates are given in 4.4.4.

### 4.3 Laboratory preparation of media

#### 4.3.1 General

The accurate preparation of culture media is one of the fundamental steps in microbiological examination and it shall be given special care.

Respect good laboratory practice and the manufacturer's instructions regarding the handling of dehydrated media and other components, particularly those containing hazardous materials, i.e. bile salts or other selective agents.

Where media are prepared from dehydrated commercial formulations, follow the manufacturer's instructions precisely. Document all relevant data, i.e. mass/volume, pH, date of preparation, sterilization conditions, operator.

For media prepared from individual components, follow the recipe precisely and record all details and, in addition, the full identity (i.e. code and batch number) of all the components used.

#### 4.3.2 Water

The conductivity of water produced in the laboratory shall be no more than  $25\text{ }\mu\text{S cm}^{-1}$  (equivalent to a resistivity  $\geq 0,4\text{ M}\Omega\text{ cm}$ ) at  $25\text{ °C}$ , unless otherwise required by design.

Microbial contamination should not exceed  $10^3\text{ ml}^{-1}$  and should preferably be below  $10^2\text{ ml}^{-1}$ . A regular verification of microbial contamination should be established according to ISO 6222<sup>[1]</sup> (with an incubation at  $22\text{ °C}$  for  $68\text{ h} \pm 4\text{ h}$ ) or an equivalent validated method.

#### 4.3.3 Weighing and rehydration

Carefully weigh the appropriate amount of dehydrated medium (taking care not to inhale powder, especially with media containing toxic substances) and progressively mix with the required amount of water to avoid clumping.

#### 4.3.4 Dissolution and dispersion

Dehydrated media need rapid dispersion by instant and repeated or continuous stirring followed by heating, if necessary, to dissolve. Media containing agar should be allowed to soak for several minutes prior to heating while mixing to dissolve.

#### 4.3.5 Measurement and adjustment of pH

Measure the pH using a pH-meter and adjust before sterilization, if necessary, so that, after sterilizing and cooling to 25 °C, the medium is at the required pH within  $\pm 0,2$  pH units, unless otherwise stated. The adjustment is normally carried out using a sodium hydroxide solution of approximately 40 g/l [ $c(\text{NaOH}) \approx 1 \text{ mol/l}$ ] or dilute hydrochloric acid of approximately 36,5 g/l [ $c(\text{HCl}) \approx 1 \text{ mol/l}$ ]. If adjustment is performed after sterilization, use a sterilized solution.

NOTE Commercially manufactured media can show significant changes in pH before and after autoclaving. However, provided distilled or deionized water is used, pH adjustment prior to autoclaving is not necessary.

#### 4.3.6 Dispensing

Dispense the medium into appropriate containers having a volume at least 20 % greater than that of the medium.

#### 4.3.7 Sterilization

##### 4.3.7.1 General

The sterilization of culture media and of reagents is generally carried out using sterilization by moist heat (4.3.7.2) or sterilization by filtration (4.3.7.3).

Certain media do not need sterilization by autoclaving but can be used following boiling. For example, media for Enterobacteriaceae containing brilliant green are particularly sensitive to heat and light and should be rapidly cooled after boiling and protected from strong light. Also, some reagents can be used without sterilization (refer to the appropriate International Standard or manufacturer's instructions).

##### 4.3.7.2 Sterilization by moist heat

See ISO 7218.

Sterilization by moist heat is performed in an autoclave or media preparator. Generally, the autoclaving operation takes 15 min at  $121 \text{ °C} \pm 3 \text{ °C}$ . For volumes greater than 1 000 ml, adapt the sterilization cycle as necessary. In all cases, follow the instructions given in the International Standard or the manufacturer's instructions.

NOTE Overheating can occur when large volumes of media (> 1 000 ml) are processed in an autoclave. See ISO 7218.

After heating it is essential that media be cooled in a manner that prevents boiling over. This is particularly important for media in large volumes and for sensitive media, e.g. media containing brilliant green.

##### 4.3.7.3 Sterilization by filtration

Sterilization by filtration can be performed under vacuum or pressurized conditions. Use sterile equipment and membranes with a pore diameter of 0,2  $\mu\text{m}$ . Sterilize the different parts of the filtration apparatus according to ISO 7218 or use pre-sterilized equipment.

Some filter membranes may retain proteins or other substances (such as antibiotics). In order to obtain the correct concentration, the user should pre-wet the filter.

##### 4.3.7.4 Checking

After autoclaving, boiling or filtration, all media should be checked, in particular with respect to pH, colour, sterility and physical consistency.

#### 4.3.8 Preparation of supplements

**CAUTION** — Manufactured supplements containing toxic agents, particularly antibiotics, shall be handled with care avoiding dispersion of powder which can give rise to allergic or other reactions in laboratory personnel. Take appropriate precautions and follow the manufacturer's instructions when making solutions.

Do not use manufactured supplements beyond their stated shelf-life which, for antibiotic working solutions, is generally the same day. Under certain circumstances, antibiotic solutions may be stored frozen in suitable aliquots but should not be re-frozen after thawing. The potential loss of activity due to freezing should be established with the manufacturer or tested by the user.

### 4.4 Preparation for use

#### 4.4.1 Melting of agar culture media

Melt a culture medium by placing it in a boiling water bath or by any other process which gives identical results (e.g. a steam flow-through autoclave). Media that have previously been autoclaved should be reheated for a minimum time to maintain media quality. Avoid overheating and remove when it has melted. Stand at room temperature for a short time, e.g. 2 min, to avoid glass breakage.

Cool the molten medium to a temperature ranging from 47 °C to 50 °C in a thermostatically controlled water bath. The time needed to reach 47 °C to 50 °C depends on the type of medium, the volume and the number of units in the water bath. Molten medium should be used as soon as possible, but it is recommended that it not be retained for more than 4 h. Unused medium shall not be resolidified for subsequent use. In the case of particularly sensitive media, the holding time of molten media shall be shortened, as specified in the relevant International Standard.

Establish and document an agar tempering regime by setting a thermometer into agar medium in a separate container similar to that used for the test medium.

Media to be added to the sample should be tempered between 44 °C and 47 °C, or as specified in the relevant International Standard.

#### 4.4.2 De-aeration of culture media

If necessary, just prior to use, heat the culture medium in boiling water or under a flow of steam for 15 min, with lids or caps loose; after heating, tighten the caps and cool down rapidly to the operating temperature.

#### 4.4.3 Addition of supplements

Heat-labile supplements should be added to the medium after it has been cooled to a temperature of 47 °C to 50 °C. Allow the sterile supplement to come to room temperature before adding it to the agar medium. Cold liquids may cause agar to gel or form transparent flakes. Mix all supplements into the medium gently and thoroughly, then distribute into the final containers as quickly as possible.

#### 4.4.4 Preparation and storage of media in Petri dishes

Pour the molten agar culture medium into Petri dishes so as to obtain a thickness of at least 3 mm (for 90 mm diameter dishes, 18 ml to 20 ml of agar are normally required) or as specified in the relevant International Standard. Allow the agar to cool and solidify by placing the plates with lids in place on a cool, horizontal surface. If plates are stored or if incubation is extended beyond 48 h, or is above 40 °C, more culture medium is required.

**NOTE** During incubation, a loss of moisture of the agar media occurs, which can affect the growth of microorganisms in some circumstances. Factors influencing water loss are medium composition, amount of medium in the plates, the type of incubator, i.e. fan-assisted or otherwise, humidity of the atmosphere in the incubator, the position and number of the plates in the incubator and the incubation temperature.

Use the solidified medium immediately or store under conditions which prevent its composition from being modified, i.e. in the dark and/or in the refrigerator at  $5\text{ °C} \pm 3\text{ °C}$  in sealed bags (see 4.2). Label the plates on the base or on the side with the date of preparation, and/or expiry date and identity. Alternative coding systems meeting these requirements may be used.

The shelf-life of poured plates will increase if they are stored in sealed plastic bags. In order to avoid the occurrence of condensate, the plates shall be cool before being placed into bags. Do not dry the surface of agar plates prior to chill storage.

In general, for the surface inoculation of a solid culture medium, dry the plates, preferably with the lids removed and with the agar surface facing downwards, in an oven set at a temperature between  $25\text{ °C}$  and  $50\text{ °C}$  or in a laminar-flow cabinet, until the droplets have disappeared from the surface of the medium. Do not overdry them. Commercially prepared ready-to-use agar plates should be stored and used according to the manufacturer's instructions.

#### 4.5 Disposal of media

Both contaminated and unused media shall be disposed of in a manner that is safe and meets any local or national regulations.

### 5 Preservation and maintenance of control strains

#### 5.1 General

There are several methods available, e.g. lyophilization, storage on beads at  $-70\text{ °C}$ , or using liquid nitrogen, for the successful preservation and maintenance of all microorganisms relevant to food and water microbiology. A single method may not be appropriate for all strains.

A flowchart of maintenance and preparation is given in Annex B.

#### 5.2 Control strains from commercial sources

If control strains are obtained from reference collections or commercial suppliers holding ISO 9000<sup>[3]</sup> certification or other appropriate certification and maintained in their original containers, the manufacturer's directions for their cultivation and use shall be followed.

#### 5.3 Laboratory-prepared reference stocks

Stock cultures of reference strains (see Clause B.1) for performance-testing purposes shall be maintained and handled in a manner that minimizes the opportunity for cross-contamination, mutation or alteration of typical characteristics. Reference stocks should be stored in multiple aliquots, usually either deep frozen ( $\leq -70\text{ °C}$ ) or lyophilized. At a higher temperature, storage time should be reduced.

Their growth characteristics should be fully documented for each medium on/in which they will be utilized as test organisms.

Reference stocks shall not be used to prepare reference strains.

#### 5.4 Stock cultures

Stock cultures are usually prepared from lyophilized or deep-frozen reference stocks (see Clause B.2). Aliquots shall be handled in a manner that avoids possible cross-contamination of the reference stock and/or its deterioration. Stock cultures should be prepared by resuspending an aliquot of the reference stock in a non-selective growth medium and incubating to yield a stationary phase culture.

For commercially available preservation systems, the manufacturer's instructions shall be rigorously followed.

Stock cultures shall not be used to prepare reference strains or reference stocks.

## 5.5 Working cultures

Working cultures are prepared from stock cultures or reference stocks.

Working cultures shall not be used to prepare reference strains, reference stocks or stock cultures.

## 6 Performance testing of finished culture media

### 6.1 General

Performance-testing procedures are specified in ISO/TS 11133-2.

Minimal guidelines are given in 6.2 and 6.3; in practice, foods and water may contain stressed microorganisms. The suitability of the medium with respect to the recovery of stressed cells should be taken into account.

### 6.2 Physical quality control

See ISO/TS 11133-2.

### 6.3 Microbiological quality control

#### 6.3.1 Contamination

An appropriate amount of each batch should be tested for contamination.

#### 6.3.2 Test organisms

A set of test organisms should only contain microorganisms with stable characteristics representative of their species and that have been shown to be reliable for the demonstration of optimal performance of a particular laboratory-prepared medium. The test organisms should primarily comprise strains that are widely available in reference culture collections, but well-characterized strains isolated by the laboratory may also be included. The relevant cultural characteristics of the reference stock should be examined and recorded by the laboratory or a new strain chosen if atypical characteristics occur. It is preferable to use strains that have originated from foods or water although not all culture collections provide such data on their origin.

The test microorganisms for each medium may include:

- robust positive strains with typical characteristics;
- weakly positive strains (i.e. of a more sensitive nature);
- strains showing negative characteristics;
- partly or completely inhibited strains.

Suitable test microorganisms are listed in ISO/TS 11133-2:2003, Annex B.

NOTE Reference [6] describes a validated collection of test strains for media evaluation.

### 6.3.3 Ready-to-use media and reagents

Manufacturers of commercially available ready-to-use media, especially if accredited to ISO 9000<sup>[3]</sup>, shall have a quality programme in place and may issue a quality certificate with the media they supply. Under those conditions, the user may not need to carry out extensive testing on such media but should ensure that storage conditions are maintained as recommended by manufacturers. For ready-to-use media to which supplements have been added, at least a qualitative test is recommended.

### 6.3.4 Media prepared from commercially available dehydrated formulations

For isolation and enumeration media, at least semi-quantitative testing should be performed. For characterization test media, qualitative testing may be sufficient. Quantitative tests give greater assurance of media quality.

For those media which contain no indicators or selective agents, the use of a single positive test strain is appropriate. For those media which do contain indicators or selective agents, strains which demonstrate the function of the indicator(s) and selectivity shall be utilized. For complex media, i.e. with added supplements, each batch should be verified with strains that have the characteristics listed in 6.3.2.

### 6.3.5 Media prepared from basic individual components

It is recommended that, in addition to the qualitative tests described in 6.3.4, quantitative testing be performed in order to monitor trends in quality of basic materials, productivity of the medium and in-laboratory production protocols.

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## Annex A (informative)

### Designation of the components of the culture media in standards on microbiological analysis of food and animal feeding stuffs

#### A.1 General

A harmonized description of the various components in the composition of culture media in microbiological standard methods is given in Clauses A.2 to A.5.

#### A.2 Peptones

- Enzymatic digest of casein<sup>1)</sup>
- Enzymatic digest of soybean meal
- Enzymatic digest of animal tissues<sup>2)</sup>
- Enzymatic digest of heart
- Enzymatic digest of gelatin
- Enzymatic digest of animal and plant tissue<sup>3)</sup>

#### A.3 Extracts

- Meat extract
- Brain-heart extract
- Yeast extract
- Ox bile for bacteriology
- Bile salts
- Bile salts No.3

#### A.4 Agar

- Bacteriological agar

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1) This includes peptic digest of casein, tryptic digest of casein and tryptone.

2) This includes meat peptone, peptic digest of meat, pancreatic digest of meat.

3) This includes tryptose.

## A.5 Other

- Egg yolk emulsion
- Skim milk powder
- Acid hydrolysate of casein

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