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## Introduction

This draft standard is based on national and international discussions. Your comments on this draft are invited and will assist in the preparation of the consequent standard.

For international standards, comments will be reviewed by the relevant UK national committee before sending the consensus UK vote and comments to the international committee, which will then decide appropriate action. If the international standard is approved, it is usual for the text to be published as a British Standard.

For national standards, comments will be reviewed by the relevant UK national committee and the resulting standards published as a British Standard.

## UK Vote

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## Template for comments and secretariat observations

|                  |                        |
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| 1  | 2                                          | (3)                          | 4               | 5                                                                                                                                  | (6)                                                                      | 7                                                  |
|----|--------------------------------------------|------------------------------|-----------------|------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|----------------------------------------------------|
| MB | Clause No./ Subclause No./Annex (e.g. 3.1) | Paragraph/ Figure/Table/Note | Type of comment | Comment (justification for change) by the MB                                                                                       | Proposed change by the MB                                                | Secretariat observations on each comment submitted |
|    | 3.1                                        | Definition 1                 | ed              | Definition is ambiguous and needs clarifying.                                                                                      | Amend to read '...so that the mains connector to which no connection...' |                                                    |
|    | 6.4                                        | Paragraph 2                  | te              | The use of the UV photometer as an alternative cannot be supported as serious problems have been encountered in its use in the UK. | Delete reference to UV photometer.                                       |                                                    |



# DRAFT International Standard

## ISO/DIS 11133

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

ICS: 07.100.30; 07.100.20

ISO/TC 34/SC 9

Secretariat: **AFNOR**

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Voting terminates on:  
**2025-09-15**

Member bodies are requested to consult relevant national interests in ISO/TC 147/SC 4 before casting their ballot to the e-Balloting application.

This document is circulated as received from the committee secretariat.

## ISO/CEN PARALLEL PROCESSING

Reference number  
ISO/DIS 11133:2025(en)

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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.

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](http://www.iso.org/directives)

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

This document was prepared by Technical Committee ISO/TC 34, Food products, Subcommittee SC 9, Microbiology, in collaboration with Technical Committee ISO/TC 147, Water quality, Subcommittee SC 4, Microbiological methods, and in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 463, Microbiology of the food chain, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Any feedback or questions on this document should first be directed to the user's national standards body. A complete listing of these bodies are found at [www.iso.org/members.html](http://www.iso.org/members.html).

This second version of ISO 11133 cancels and replaces ISO 11133:2014, which has been technically revised, and incorporates the following:

Inclusion of ISO11133 Amendment 1 (2018)

Inclusion of ISO11133 Amendment 2 (2020)

Exclusion of those culture media (and their performance testing details) which have been now detailed within individual Standards. Refer to <https://committee.iso.org/sites/tc34sc9/home/generalstandards/content-left-area/culture-media/iso-11133-on-culture-media.html>

Amalgamation of [Annexes E](#), [F](#) and [K](#) for those media and reagents not yet detailed within individual Standards

Inclusion of new [Annex F](#) (normative) *Reference culture media*

[Annex G](#) is now Informative, with new content

Inclusion of new [Annex K](#) (informative) *Guideline for the preparation of standardized test suspensions* (inocula) and preservation by ultra-low freezing

Inclusion of new [Annex L](#) (informative) *pH measurement of culture media*

Inclusion of new [Annex M](#) (informative) *Guideline for the evaluation of buffered peptone water (BPW)*

Inclusion of performance testing for contact plates

## ISO/DIS 11133:2025(en)

Inclusion of distinguishable target strains

Inclusion of other amendments and improvements as determined through technical committee working groups

## 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 culture media can be specific to both the sample and the microorganisms 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 culture medium,
- b) that the culture medium is "fit for purpose", and
- c) that the culture 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 culture 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.

Culture Media and reagents: type and criteria vs section of this Standard

| Culture media and reagents type                  | Criteria and section of Standard |         |             |         |             |       |
|--------------------------------------------------|----------------------------------|---------|-------------|---------|-------------|-------|
|                                                  | Productivity                     |         | Selectivity |         | Specificity |       |
| Solid non- selective culture media               | Quantitative                     | 7.2.2   |             |         |             |       |
| Solid selective culture media for enumeration    | Quantitative                     | 7.2.2   | Qualitative | 7.5.1   | Qualitative | 7.5.2 |
| Solid selective culture media for isolation      | Qualitative                      | 7.5.1   | Qualitative | 7.5.1   | Qualitative | 7.5.2 |
| Solid non- selective culture media for isolation | Qualitative                      | 7.5.1   |             |         |             |       |
| Solid reference culture medium                   | Quantitative                     | 7.2.2   |             |         |             |       |
| Liquid non- selective enrichment culture media   | Qualitative                      | 8.4.2.1 |             |         |             |       |
| Liquid selective enrichment culture media        | Qualitative                      | 8.3     | Qualitative | 8.3     |             |       |
| Liquid selective culture _media for confirmation | Qualitative                      | 8.4.2.2 | Qualitative | 8.4.2.2 |             |       |
| Liquid selective culture _media for enumeration  | Quantitative                     | Annex I | Qualitative | 8.2.1.2 |             |       |
| Diluents                                         | Quantitative                     | 9.2     |             |         |             |       |
| Liquid transport media                           | Quantitative                     | 9.3.2   |             |         |             |       |
| Solid transport media                            | Qualitative                      | 9.3.3   |             |         |             |       |
| Contact plates                                   | Quantitative                     | 7.4.1   |             |         |             |       |

The widespread adoption of minimum performance criteria for culture media will 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. This includes culture media not described in International Standards, including proprietary culture media, or other methods.

In the microbiological analysis of food, animal feed, environment and water, the requirements of this International Standard have precedence in the assessment of culture media quality.

ISO 11133 was published in 2014 and superseded all previous ISO publications and information on culture media. Amendment 1 (published in 2018) provided corrections and explanations whilst Amendment 2 (published in 2020) described the performance testing of confirmation media and reagents.

## ISO/DIS 11133:2025(en)

Since 2014, many standards have been published which contain their own specifications for performance testing of culture media used in the standard. These specifications are based on ISO 11133 but for technical reasons, they may include new control strains and/or revised specifications for the

performance testing. All new and revised standards now specify the performance testing of culture media and reagents including the specification of control strains in the standard itself.

Any information on performance testing of culture media and reagents described in new or revised standards published since 2014 takes precedence over ISO 11133.

When specific standards are revised and new standards developed, they will include a paragraph for performance testing of the culture media used in the standard, and will refer to ISO 11133 for detailed methods (or detailed protocols). To ensure performance testing is to the latest requirements, the ISO webpage enables a rapid crosscheck between this Standard and the individual food and water Standards published. Please refer to the link <https://committee.iso.org/sites/tc34sc9/home/general-standards/content-left-area/culture-media/iso-11133-on-culture-media.html>, where the spreadsheet link shown will be updated at least annually.



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

## 1 Scope

This International Standard defines terms related to quality assurance of culture media and reagents and specifies the requirements for the preparation of culture media and reagents 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.

These requirements are applicable to all categories of culture media and reagents prepared for use in laboratories performing microbiological analyses.

This document also sets criteria and describes methods for the performance testing of culture media and reagents.

The document is applicable to end-users, commercial bodies, non-commercial bodies and labs preparing their own media.

This covers all formats of culture media and reagents.

The principles covered in this Standard, as described above, can be equally applied to the preparation, storage and performance testing of culture media and reagents (used in the intended analysis as described above) that are not captured in international Standards; this includes proprietary culture media, or other methods.

The criteria for the performance of those culture media and reagents will be described within those other methods or in the manufacturers' certificates.

## 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, *(parts 1-5), Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination (including amendments)*

ISO 7704, *Water quality — Requirements for the performance testing of membrane filters used for direct enumeration of microorganisms by culture methods*

ISO 7218, *Microbiology of the food chain — General requirements and guidance for microbiological examinations*

ISO 8199, *Water quality — General requirements and guidance for microbiological examinations 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.

## 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 or reagent**

#### **lot of culture medium or reagent**

homogeneous and fully traceable unit of a culture 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

Note 1 to entry: This also applies to units produced in or by the user lab

Note 2 to entry: This applies to the unit produced, whether from individual components or from commercial blend.

### 3.1.3

#### **chromogenic substrate fluorogenic substrate**

substrate containing a chromophore/fluorophore group and a substrate utilizable by microorganisms

Note 1 to entry: After splitting the chromogenic/fluorogenic substrate, the chromophore is released, and a coloured end-product is visible/detectable, or a fluorophore is released and is visible/detectable using ultraviolet (UV) light.

## 3.2 Terminology of performance testing

### 3.2.1

#### **performance of culture medium or reagent**

response of a culture medium or reagent to a challenge by test microorganisms under defined conditions

### 3.2.2

#### **target microorganism**

microorganism or group of microorganisms to be detected or enumerated which can be expected to grow under defined conditions

### 3.2.3

#### **non-target microorganism**

microorganism that is suppressed by the culture medium and/or conditions of incubation or does not show expected characteristics of the target microorganism

### 3.2.4

#### **productivity of culture medium**

a measure of the 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

#### **electivity of culture medium**

#### **specificity of culture medium**

demonstration, under defined conditions, that non-target microorganisms, if able to grow on the culture medium, 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

#### 3.3.2 Culture media classified by composition

##### 3.3.2.1

##### **chemically defined culture 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 culture 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 culture 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 microorganisms, 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 culture medium**

culture medium consisting of an aqueous solution of one or more constituents, such as buffered peptone water

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 culture media in tubes, flasks or bottles are commonly called “broths”.

##### 3.3.3.2

##### **solid culture medium**

##### **semi-solid culture medium**

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

Note 1 to entry: Due to the worldwide use of culture media solidified with agar-agar, the shortened term “agar” is often used synonymously for solid culture media and therefore in connection with nouns, e.g. “Plate Count agar”.

Note 2 to entry: Solid culture media poured into Petri dishes are commonly called “plates”. Solid culture media poured into tubes or small bottles that are kept in slanted positions while the culture media are solidifying are often called “slants” or “slopes”. If the culture 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 can 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 culture medium**

culture 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 culture medium can also be used as a pre-enrichment culture medium, e.g. buffered peptone water.

#### 3.3.4.5

##### **pre-enrichment culture medium enrichment culture medium**

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

EXAMPLE Buffered peptone water

#### 3.3.4.5.1

##### **selective enrichment culture medium**

enrichment culture 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); Half-Fraser broth

#### 3.3.4.5.2

##### **non-selective enrichment culture medium**

enrichment culture medium that allows the growth of a wide variety of microorganisms

EXAMPLE Heart infusion broth

#### 3.3.4.6

##### **isolation culture medium**

solid or semi-solid culture medium that allows the growth of microorganisms with the formation of distinct colonies.

#### 3.3.4.6.1

##### **selective isolation culture medium**

isolation culture medium that allows growth of specific target microorganisms, while inhibiting, totally or partially, other microorganisms

EXAMPLES Modified semi-solid Rappaport- Vassiliadis (MSRV) agar,

Modified charcoal cefoperazone deoxycholate agar (mCCD agar)

#### 3.3.4.6.2

##### **non-selective isolation culture medium**

isolation culture medium that is not intended to selectively inhibit microorganisms

EXAMPLE Nutrient agar

#### 3.3.4.6.3

##### **chromogenic selective culture medium fluorogenic selective culture medium**

chromogenic/fluorogenic culture medium that also contains selective compounds which inhibit, totally or partially, accompanying flora occurring in test materials and thus support the targeted detection of microorganisms

EXAMPLE Agar Listeria according to Ottaviani and Agosti; modified lauryl sulfate tryptose broth (LST + MUG); chromogenic coliform agar (CCA); MUG/EC medium (4-methylumbelliferyl- $\beta$ -D glucuronide /EC medium)

#### 3.3.4.7

##### **differential culture medium characterization culture medium**

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

EXAMPLE Tryptone bile X-glucuronide (TBX) agar; Xylose lysine deoxycholate (XLD) agar, blood agar

Note 1 to entry: Differential culture media that can be used as isolation culture media are referred to as isolation/differential culture media, e.g. Xylose lysine deoxycholate (XLD) agar.

#### 3.3.4.8

##### **identification culture medium**

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

EXAMPLE Rabbit plasma fibrinogen agar (RPFA); Tryptone bile X-glucuronide (TBX) agar

#### 3.3.4.9

##### **enumeration culture medium**

selective or non-selective culture medium for quantitative methods

EXAMPLE Baird-Parker agar; Yeast extract agar

#### 3.3.4.10

##### **confirmation culture medium**

Culture medium that contributes to the identification or characterization of the microorganism following a preliminary resuscitation and/or enrichment and/or isolation step

EXAMPLE Triple sugar iron (TSI) agar

#### 3.3.4.11

##### **culture medium containing neutralizers**

transport medium, dilution medium or culture medium containing neutralizing ingredients to inactivate detergents/disinfectants or other biocidal agents

#### 3.3.4.12

##### **Multipurpose culture medium**

Culture medium assigned to several categories

EXAMPLE Blood agar is a resuscitation culture medium according to [3.3.4.4](#), an isolation culture medium according to [3.3.4.6](#) and a differential culture medium according to [3.3.4.7](#) used for detection of haemolysis. Buffered peptone water is a diluent according to [3.3.4.3](#), a resuscitation culture medium according to [3.3.4.4](#), and a pre-enrichment culture medium according to [3.3.4.5](#).

**3.3.4.13****reference culture medium**

*culture medium* ([3.3.1](#)), usually non-selective, for determination of the *reference count* ([3.4.6](#))

Note 1 to entry: The reference culture medium is usually a non-selective *culture medium* ([3.3.1](#)), which is different to the culture medium under test and has been demonstrated to be suitable for use in the performance testing.

EXAMPLE Tryptone soya agar (TSA); Sabouraud dextrose agar (SDA)

**3.3.5 Culture media classified according to preparation method****3.3.5.1****ready-to-use culture medium**

liquid, solid or semi-solid culture medium that is supplied in plates, bottles, tubes or other containers, in ready-to-use form; or, ready-to-use *after re-melting*; or, ready-to-use *after re-melting and supplementing*

**3.3.5.1.1****finished culture medium**

Culture medium in a form that is ready for inoculation

**3.3.5.1.2****semi-finished culture medium**

Culture medium which requires remelting or remelting and supplementing to form *ready-to-use culture medium after remelting* ([3.3.5.1.3](#)) or *ready-to-use culture medium after remelting and/or supplementing* ([3.3.5.1.4](#))

**3.3.5.1.3****ready-to-use culture medium after remelting**

Culture medium to be remelted, for instance for use in the pour-plate technique or to be poured into Petri dishes

**3.3.5.1.4****ready-to-use culture medium after remelting and/or supplementing**

Culture medium to be remelted, supplemented, or be remelted and supplemented, and dispensed before use (incomplete ready-to-use culture medium)

EXAMPLE Tryptose sulphite cycloserine (TSC) agar; Baird-Parker or Rabbit Plasma Fibrinogen (RPF) agar; Fraser/Half-Fraser broth

**3.3.5.2****culture medium prepared from commercially dehydrated formulations**

culture medium in dry form which requires rehydration and processing before use, resulting in one of two kinds of culture media:

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

EXAMPLE Powders, compacted granules, lyophilized products

**3.3.5.3****Culture medium prepared from individual components**

Culture medium produced by a microbiology laboratory entirely from its individual ingredients

**3.4 Terminology for test microorganisms****3.4.1****test microorganism****also named control microorganisms or control strains**

microorganism generally used for performance testing of culture media

Note 1 to entry: Test microorganisms are further defined according to their source (see [3.4.2](#) to [3.4.7](#)).

### 3.4.2

#### **reference strain**

microorganism obtained directly from a reference culture collection and defined to at least the species level

Note 1 to entry: A reference culture collection is a culture collection which is a member of the World Federation of Culture Collections (WFCC) or the European Culture Collections' Organization (ECCO).

Note 2 to entry: A **reference strain** is catalogued and described according to its characteristics and preferably originating from food, animal feed, the food or feed production environment or water as applicable

### 3.4.3

#### **reference stock**

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

### 3.4.4

#### **stock culture**

primary subculture from a reference stock

### 3.4.5

#### **working culture**

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

### 3.4.6

#### **reference count**

inoculum level total count of colonies on a *reference culture medium* ([3.3.4.13](#))

### 3.4.7

#### **reference material RM**

microbiological material containing a quantity of revivable microorganisms, sufficiently homogenous and stable with respect to the quantity of revivable microorganisms, which has been established to be fit for its intended use in a measurement process

Note 1 to entry: See ISO Guide 30[3].

Note 2 to entry: For in-house prepared quality control reference materials (QRMs), often so-called "in-house reference materials" or "internal RM", see ISO/TR 33402[103].

### 3.4.8

#### **certified reference material CRM**

microbiological *reference material* ([3.4.7](#)) characterized by a metrologically valid procedure for the quantity of revivable microorganisms

Note 1 to entry: See ISO Guide 30[3].

Note 2 to entry: Metrologically valid procedures for the production and certification of RMs are given in, among others, ISO 17034[94], ISO 33401[104] and ISO 33405[105].

Note 3 to entry: A microbiological CRM is accompanied by a certificate that provides the value of the specified quantity of revivable microorganisms, its associated uncertainty and a statement of metrological traceability. A CRM is certified only for the method/methods and media that were included in the certification process, otherwise its function is like an (ordinary) *reference material* ([3.4.7](#)).

### 3.4.9

#### **passage**

transfer of microorganism, from a viable culture to fresh medium, with successful growth of the microorganism.

Note 1 to entry: Any form of sub-culturing is considered to be a transfer/passage.

### 3.5 Terminology for reagents and supplements

#### 3.5.1

##### **reagent**

a compound or mixture added to a known culture medium to start or test a biochemical reaction

#### 3.5.2

##### **Confirmatory reagent**

a compound or mixture, when added to a known culture medium to start or test a biochemical reaction, where the identity of the organism is already presumptively known from earlier results

#### 3.5.3

##### **Supplement**

ingredient or ingredients which are necessary to the formula of a given medium, but are not included in the initial dehydrated formulation; and are added prior to, or at the time of, final dispense, or prior to use.

EXAMPLE defibrinated blood; antibiotics; egg yolk emulsion

## 4 Quality assurance management

### 4.1 Documentation

#### 4.1.1 Documentation from manufacturer or producer

The following information shall be available from the manufacturer or producer (commercial or non-commercial bodies supplying culture media or reagent to third parties):

- name of the culture medium or reagent, individual components and any supplements and, if possible, their product codes;
- technical data sheet, e.g. formulation, intended use, filling quantity if applicable, references;
- safety and/or hazard data where needed;
- date of production;
- batch number and expiry date;
- target pH of the complete culture medium at time of manufacture or preparation;
- storage information;
- assigned shelf-life;
- quality control certificate showing test microorganisms used and results of performance testing with criteria of acceptance.

#### 4.1.2 Delivery acceptance of products

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

- identification of the product;
- integrity of packaging received, transportation and in transit storage;
- expiry date of the product;
- documentation supplied;
- number of units received.

Record the date of receipt.

## 4.2 Storage

### 4.2.1 General

In all cases, follow the manufacturer's instructions.

### 4.2.2 Quality management and product control of dehydrated culture media and supplements

Culture media are delivered as dehydrated powders or in compacted granular form in sealed containers. Supplements of different selective or diagnostic substances are supplied in either the lyophilized, powder or liquid state. Plan purchases to encourage a regular turnover of stock (i.e. first in, first out). When a new container is opened

- check the seal,
- record date of first opening, and
- visually assess the contents of opened containers (see below).

After opening a new container, the quality of the culture medium will depend on the storage environment. Loss of quality of dehydrated culture media is shown by change in flow characteristics of the product, homogeneity, caking, colour changes etc. Discard any dehydrated culture medium that has absorbed moisture or shows obvious changes in physical appearance.

When a container of dehydrated culture medium is opened, date the container and indicate a maximum storage time.

### 4.2.3 Quality management and product control of ready-to-use culture media

Follow the manufacturer's instructions.

Users of ready-to-use culture media should undertake a brief inspection of the media on receipt in their laboratory.

Examination should include:

- Integrity of packaging.
- Broken or cracked petri dishes/bottles/tubes.
- Quality and accuracy of labelling.
- Expiry date.
- Dehydration.
- Discolouration.
- Sloped or uneven filling of Petri dishes.
- Contamination.
- Crystalline pattern on surface of medium (indicative of freezing).
- Presence of bubbles.
- Presence of leakage.

### 4.2.4 Quality management and product control of reagents

Establish the expiry date for stored reagents by checking after defined storage times for their physical, chemical and microbiological performance characteristics as described in this International Standard.

### 4.3 Laboratory preparation of culture media and reagents

#### 4.3.1 General

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

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

When a formula indicates an ingredient in hydrated form (e.g.  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  for BPW in ISO 6887-1) it can be replaced by an anhydrous or hydrated ingredient with a different number of water molecules, as long as the final quantity of the ingredient takes account of this difference by calculation of the molar mass.

When culture media or reagents are prepared from dehydrated commercial formulations, follow the manufacturer's instructions. Document all relevant data, e.g. code, lot number, mass/volume, pH, date of preparation, sterilization conditions, operator, and any added supplements.

For culture media or reagents prepared from individual components, follow the formulation accurately, within 2 % of the required amounts (ISO7218, ISO8199). Record all details as before and, in addition, the full identity (i.e. code, lot number and expiry date if available) of all the components used.

[Annex D](#) gives an example of a record card for this information.

#### 4.3.2 Quality of basic culture medium components

Formulation of basic culture media components is described in the specific International Standards (see the Bibliography). When available, the molecular mass and the CAS<sup>1)</sup> number of a chemical substance should be stated in the formulation.

It is sometimes the case that a particular ingredient (for example those listed below) specified in the formulation has to be modified to achieve constant and consistent performance of the culture medium.

- peptones and meat or yeast extracts variable in their nutritive properties;
- agar variable in its gelling properties;
- buffering substances;
- bile salts, bile extract and deoxycholate, antibacterial dyes, depending on their selective properties;
- indicator dyes;
- antibiotics, depending on their activity and interactions with other ingredients.

**NOTE** On an industrial scale, manufacturers usually state that the formulation will be optimized to meet the required performance criteria. It is common practice to first select the ingredient, then adjust the concentration between lots to achieve the same performance and to minimize batch-to-batch variations.

#### 4.3.3 Water

For the preparation of culture media, supplements and reagents, use only purified water; i.e. distilled, demineralized, deionized or produced by reverse osmosis, or of equivalent quality; free from substances likely to inhibit or influence the growth of the microorganisms under the test conditions e.g. traces of chlorine, traces of ammonia and traces of metal ions.

1) CAS Number/CAS Registry Number: a unique numerical identifier of the Chemical Abstracts Service (CAS) for chemical elements, compounds, polymers, biological sequences, mixtures and alloys.

Store the purified water in tightly closed containers made from an inert material (neutral glass, polyethylene, etc.) that are free from all inhibitory substances. It is however recommended that the water is used as soon as produced.

Microbial contamination should not exceed  $10^3$  colony forming units (cfu) /ml and preferably be below  $10^2$  cfu /ml. Microbial contamination should be regularly monitored according to ISO 6222[4] with an incubation at  $22\text{ °C} \pm 2\text{ °C}$  for  $68\text{ h} \pm 4\text{ h}$  or using an equivalent method (e.g., using plate count agar (PCA) instead of yeast extract agar (YEA)).

NOTE Water which has been passed through an ion exchanger (demineralized) can have a very high microorganism content; it is therefore advised not to use this process without checking the microbial content of the water. Consult the manufacturer in order to find out the best means of minimising microbial contamination. Highly contaminated demineralized water, even sterilized by filtration, can still contain substances that are inhibitory for the growth of certain microorganisms.

The conductivity of water used in the laboratory shall be no more than  $25\text{ }\mu\text{Scm}^{-1}$  (equivalent to a resistivity  $\geq 0,04\text{ M}\Omega\text{ cm}$ ) and preferably below  $5\text{ }\mu\text{Scm}^{-1}$  at ambient temperature, unless otherwise required by design. The conductivity of the water should be checked before use.

#### 4.3.4 Weighing and rehydration

Following the appropriate safety precautions, carefully weigh the required amount of dehydrated culture medium or individual ingredients and progressively mix with the required amount of water to avoid formation of lumps. Use a balance of sufficient resolution; the maximum permissible errors shall be as given in ISO 7218 and ISO 8199. Unless otherwise stated, the ingredients are added to the volume of water specified, rather than making up to that volume.

#### 4.3.5 Dissolution and dispersion

Dehydrated culture media needs to be rapidly dispersed or dissolved before autoclaving. This may be achieved by immediate and repeated, or continuous, stirring; this may also require heating with mixing to fully dissolve. Dispense into smaller volumes, where required, before autoclaving. Control any heating to avoid overheating the culture medium

#### 4.3.6 Measurement and adjustment of pH

Refer to [Annex L](#).

#### 4.3.7 Dispensing

Dispense the culture medium into appropriate containers ensuring that sufficient headspace is left to avoid boiling over during the cooling process after heat treatment by autoclaving or remelting or overflowing after addition of supplements.

NOTE This headspace might not be necessary if the pressure in the autoclave is maintained during the cooling process.

#### 4.3.8 Sterilization

##### 4.3.8.1 General

Sterilize the prepared culture media on the day of preparation.

The sterilization of culture media and of reagents is generally carried out by moist heat ([4.3.8.2](#)) or by filtration ([4.3.8.3](#)).

Certain culture media should not be autoclaved but are used following boiling. For example, culture media for *Enterobacteriaceae* containing brilliant green are particularly sensitive to heat and light; cool rapidly after boiling and protect from direct sunlight. Some reagents can be used without sterilization. In all cases, refer to the appropriate International Standard or the manufacturer's instructions.

#### 4.3.8.2 Sterilization by moist heat

Sterilization by moist heat is performed in an autoclave or culture media preparator.

It is important to ensure that autoclave cycles are set appropriately to achieve sterilization of the volume of individual containers and the number of containers in a sterilization load.

Adapt the sterilization cycle of the autoclave as necessary to ensure adequate heat treatment.

In all cases, follow the instructions given in the appropriate International Standard or the manufacturer's instructions.

After heating, it is essential that culture media are cooled in a manner to prevent boiling over or prolonged heat treatment. This is particularly important for culture media in large volumes and for culture media containing heat sensitive ingredients, e.g. culture media containing brilliant green.

Additional information on sterilization by moist heat is given in ISO 7218 and in Reference [11].

For sterilization cycles for large loads (such as those used by commercial manufacturers), see with ISO 7218.

#### 4.3.8.3 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 µm. Sterilize the filtration apparatus in accordance with ISO 8199, or use pre-sterilized equipment.

Some filter membranes might retain proteins or other substances (such as antibiotics). In order to obtain the correct concentration, choose a suitable membrane type, e.g. low protein-binding membrane, and pre-wet the filter.

#### 4.3.9 Preparation of supplements

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

Do not use beyond their stated shelf-life. Under certain circumstances, antibiotic solutions can be stored frozen in suitable aliquots but should not be re-frozen after thawing. Establish the potential loss of activity due to freezing with the manufacturer, or by testing by the user

### 4.4 Storage and shelf-life of prepared culture media and reagents

#### 4.4.1 Commercially supplied culture media and reagents

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

#### 4.4.2 Laboratory prepared culture media and reagents

##### 4.4.2.1 General

Identify all culture media and reagents in a way that ensures traceability.

The shelf-life of different culture media and reagents varies. Specific International Standards or national standards might stipulate specific conditions and shelf-life, but these shall be verified by the laboratory. The frequency of verification shall be specified by the laboratory.

Store the culture media and reagents under conditions which prevent any deterioration of their composition, namely protected from light and desiccation. If not used immediately or specified otherwise in the specific standard, store in a refrigerator at 5 °C ± 3 °C.

If refrigerated, it is generally recommended not to exceed two to four weeks of storage for plates and three to six months for sealed bottles and tubes, unless otherwise specified in specific standards or results of the laboratory shelf-life evaluation indicate a longer shelf-life. Additional information about maximum storage times for prepared culture media is given in ISO 8199, Reference [17] and Reference [21].

It is recommended that culture media and reagents to which labile supplements have been added are used on the day of preparation, unless otherwise specified in specific standards or results of laboratory shelf-life evaluation indicate a longer shelf-life is suitable (4.4.2.2). Solid culture media containing chemically reactive and/or labile substances should not be stored in bulk for remelting.

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

#### 4.4.2.2 Shelf-life evaluation

Establish the expiry date for stored culture media and reagents by checking culture media after defined storage times for their physical, chemical and microbiological performance characteristics as described in this International Standard. The frequency of verification shall be specified by the laboratory.

Check for any colour change, sign of evaporation/dehydration, change in pH or unacceptable productivity or selectivity and specificity where applicable. The expiry date shall be based on the storage time for which all the performance characteristics described above remain acceptable.

NOTE These checks are also suitable for use when commissioning commercially supplied culture media.

#### 4.4.2.3 Storage of culture media in Petri dishes

Use the solidified culture medium immediately or store solid culture media inverted and semi-solid culture media with surface upwards. Store under conditions which prevent deterioration and dehydration, i.e. in the dark and/or in the refrigerator at  $5\text{ °C} \pm 3\text{ °C}$ . Label the plates on the base or side with date of preparation and/or expiry date and identity. Alternative coding systems meeting these requirements can be used.

The shelf-life of poured plates will be prolonged by storage in sealed plastic or cellophane bags. In order to minimize condensation, the plates shall be cooled to ambient/room temperature before being placed into bags. Do not dry the surface of agar plates before chill storage.

### 4.5 Preparation for use

#### 4.5.1 Melting of agar culture media

Melt culture media by placing in a boiling water bath or by any other process which gives identical results (e.g. a steam flow-through autoclave, as specified in ISO 7218, ISO 8199). Reheat culture media that have previously been autoclaved for a minimum time to maintain culture media quality. Avoid over-heating and remove when they have melted. Stand on a heat-resistant surface at room temperature for a short time, e.g. 2 min, before putting in a water bath to cool to avoid glass breakage. Ensure water level does not cover more than  $\frac{3}{4}$  height of the bottle/container to avoid cross contamination. Loosen the caps of containers before heating and re-tighten after removal from heat.

Cool the molten culture medium to  $44\text{ °C}$  to  $47\text{ °C}$  in a thermostatically controlled water bath. The time needed to reach  $44\text{ °C}$  to  $47\text{ °C}$  depends on the type of culture medium, the volume and the number of units in the water bath. Use the molten culture medium as soon as possible; it is recommended that it is not retained for more than 4 h. In the case of particularly sensitive culture media, shorten the holding time of molten culture media; this will be specified in the relevant International Standard. Do not re-solidify or re-use unused culture media.

Establish and document an agar tempering regime by using a thermometer in agar in a separate container similar to that used for the test culture medium. This will depend on the number and size of containers in the water bath.

If culture media is not used for pour plate methods (e.g. spread plate, isolation, etc.), media can be tempered to 47 °C to 50 °C, or as specified in the International Standard. Use a water bath set at 47 °C to 50 °C.

Additional information on the use and verification of water baths is given in ISO 7218 and ISO8199.

#### 4.5.2 De-aeration of culture media

If necessary, to provide the correct air/oxygen content, heat the culture medium just prior to use 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.

#### 4.5.3 Addition of supplements

Add heat-labile supplements to the culture medium after it has been cooled to below 50 °C. If the culture medium contains agar allow the sterile supplement to equilibrate to at least room temperature before adding it to the agar culture medium. Addition of cold liquid supplements can cause agar to gel or form transparent flakes and prevent proper dispersion. Follow the manufacturer's instructions. Mix all supplements into the culture medium gently and thoroughly, then distribute into the final containers as quickly as possible.

#### 4.5.4 Preparation of solid and semi-solid culture media in Petri dishes

Pour the molten agar culture medium into Petri dishes so as to obtain a thickness of at least 3 mm (e.g. for 90 mm diameter dishes, 18 mL to 20 mL; and for 140 mm diameter dishes, 45 mL to 50 mL of agar are normally required) or as specified in the appropriate International Standard. If plates are stored, or if incubation is extended beyond 72 h, or incubation temperature is above 40 °C, more culture medium can be required per plate. Allow the agar to cool and solidify by placing the plates with lids in place on a cool, horizontal surface.

Store and use commercially prepared ready-to-use agar plates according to the manufacturer's instructions.

#### 4.5.5 Preparation of plated culture media for inoculation

For surface inoculation of solid culture media, dry the plates shortly before use until the droplets have disappeared from the surface of the culture medium. Do not over-dry the plates.

For the drying of the plates, the following points are of importance.

- The degree of humidity in culture media is important because optimum growth of bacteria will depend on the humidity conditions in and on the culture medium. Extensive moisture loss can lead, for example, to an increase in the concentrations of inhibitors in selective culture media and a reduction in the water activity at the surface of the culture medium.
- When bacteria that do not spread rapidly are cultured, and the plates look dry after acclimatization, the circumstances are such that drying is not always necessary. In that case, drying can be omitted, as it only increases the likelihood of contamination and unnecessary moisture loss.
- Select the temperature and drying time so that the likelihood of contamination is kept as low as possible and heating will not negatively affect the quality of the culture medium. The drying time will depend on the degree to which condensation is present in the Petri dish, but shall be kept as short as possible.
- In order to avoid contamination, and if the plates are not dried in a laminar-flow cabinet, plates shall always be dried with the surface of the culture medium to be inoculated turned downwards.

In practice, the plates can be dried by placing them with the agar surfaces downwards and with half- open lids in a cabinet set at a temperature of between 25 °C and 50 °C. Dry the plates until the droplets have disappeared from the surface of the culture medium. Do not dry any further. The agar plates can also be dried with the agar surface facing upwards in a laminar-flow safety cabinet (at room temperature) for 30 min to 60 min, or overnight at room temperature with the lids in place.

#### 4.6 Incubation of solid and semi-solid culture media in Petri dishes

During incubation, agar culture media will lose moisture. This can affect the growth of microorganisms in some circumstances. Factors influencing water loss are culture medium composition, amount of culture 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. Water loss can be reduced by putting the plates, in piles of up to six, in open-topped plastic bags (to avoid excessive condensation). Alternatively, the humidity of air in incubators can be increased by placing an open container of water in the bottom. Change water and disinfect containers frequently to avoid fungal contamination.

Follow the instructions given by ISO 7218 and ISO 8199 and the specific standard.

#### 4.7 Disposal of culture media and reagents

All used and unused culture media and reagents shall be disposed of in a manner that is safe and meets local and national regulations and requirements.

For more information on waste management, see ISO 7218 and ISO 8199.

### 5 Test microorganisms for performance testing

#### 5.1 General

New or revised standards should specify performance testing of culture media and reagents, including the specification of control strains and acceptance criteria, according to the requirements of [Annex J](#).

#### 5.2 Selection of test microorganisms

A set of test microorganisms should contain microorganisms with stable characteristics representative of their species, and which have been shown to be reliable for the demonstration of optimal performance of a particular prepared culture medium. The test microorganisms should primarily comprise strains that are widely available in reference culture collections, but well-characterized strains isolated by the laboratory can also be included. It is preferable to use strains which have originated from foods or water, although not all culture collections provide such information on strain origin. It is preferable to use strains that are designated Class 1 wherever possible. These strains are of lowest risk to staff conducting quality control and are also suitable for use in laboratories located in food factories.

The relevant culture characteristics of the reference stock shall be examined and recorded by the laboratory. If strain variability is encountered, investigate the possible effects of the culture medium by obtaining the same culture medium from a different manufacturer, and obtain an additional reference culture from the culture collection in which it was originally deposited.

**IMPORTANT — Users are requested to feed relevant information on strain variability and performance back to WG 5, *Culture media*, of ISO/TC 34/SC 9 through the secretariat of ISO/TC 34/SC 9.**

The test microorganisms for each culture medium or reagent should include:

- robust positive strains with typical characteristics of the target microorganism;
- weakly positive strains;
- negative strains not showing expected characteristics of the target microorganism (negative characteristics);
- partly or completely inhibited strains.

[Annex E, Table E.1](#) gives the test microorganisms to be used in specified food microbiology International Standards, and [Table E.2](#) gives the test microorganisms to be used in specified water microbiology International Standards.

[Table E.3](#) gives the test microorganisms to be used for confirmation culture media and reagents in specified food and water microbiology International Standards.

If control strains for performance testing of culture media and reagents are already specified in the International Standard, they have not been included in [Tables E.1](#), [E.2](#) or [E.3](#).

NOTE Some national restrictions and directions require the use of different serovars to those specified in these tables. Make reference to national requirements relating to the choice of *Salmonella* serovars. Refer to ISO 7218 for more information on biosafety considerations.

### 5.3 Preservation and maintenance of test microorganisms

#### 5.3.1 General

There are several methods available for the successful preservation and maintenance of all microorganisms relevant to food and water microbiology, e.g. lyophilization, storage on beads at  $-70^{\circ}\text{C}$ , or using liquid nitrogen. One method might not be appropriate for all strains. Additional methods for preservation of microorganisms are given [\[14\]](#)[\[15\]](#)[\[36\]](#)[\[37\]](#)[\[38\]](#).

Document the number of transfers of test microorganisms to prevent excessive sub-culturing that increases the risk of phenotypic alteration. One passage is defined as the transfer from a viable culture to a fresh culture medium with growth of the microorganisms. Any form of sub-culturing is considered to be a form of transfer/passage. Further information is available [\[27\]](#)[\[28\]](#)[\[35\]](#)[\[38\]](#).

See the flowcharts ([Figures B.1](#) and [B.2](#)) and additional information for maintenance and preparation given in [Annex B](#).

#### 5.3.2 Test microorganisms from commercial sources

If test microorganisms are obtained from reference collections or commercial suppliers holding ISO 17034[\[98\]](#) certification or other appropriate certification and maintained in their original containers, the manufacturer's directions for their cultivation and use shall be followed.

The laboratory shall ascertain whether the strain supplied is a reference strain or reference stock; and for reference stock, how many passages have taken place before receipt and document the information.

The laboratory shall also check that the expected characteristics are present.

#### 5.3.3 Laboratory prepared reference stocks

Maintain and handle reference stock cultures prepared from reference strains (see [Figure B.1](#)) for performance testing purposes in a manner that minimizes the opportunity for cross-contamination, mutation or alteration of typical characteristics. Verify and archive the documents received with the reference strain. Reconstitute according to manufacturer's instructions and verify the purity and characteristics of the strain before harvesting and distribution into cryotubes. Reference stocks should be stored in multiple portions, usually either deep-frozen, e.g. below  $-70^{\circ}\text{C}$ , or lyophilized. At a higher temperature, duration of viability might be reduced, and genetic modification might occur.

Document their growth characteristics for each culture medium on/in which they will be utilized as test microorganisms.

Do not use reference stocks to prepare reference strains.

#### 5.3.4 Stock cultures

Stock cultures are usually prepared from lyophilized or deep-frozen reference stocks (see [Figure B.2](#) and [5.4.2.1](#)). Handle aliquots in a manner that avoids possible cross-contamination of the reference stock and/or its deterioration. Prepare stock cultures by re-suspending a portion of the reference stock in or on a non-selective culture medium; incubate to yield a stationary phase culture.

See [5.3.1](#) for storage and documentation requirements.

Follow the manufacturer's instructions for commercially available preservation systems. Do not use stock cultures to prepare reference strains or reference stocks.

### 5.3.5 Working cultures

Working cultures are prepared from stock cultures or reference stocks and used to prepare inocula for the tests.

Do not use working cultures to prepare reference strains, reference stocks or stock cultures, or to make further working cultures.

## 5.4 Microorganisms for performance testing

### 5.4.1 General

Suitable microorganisms for routine performance testing are now mostly in the specific standards, otherwise listed in [Annex E](#).

The volumes of inocula and numbers of microorganisms used are critical; see [5.4.2.4](#) and [5.4.2.5](#).

The following guidance is given as an example of procedures suitable for producing standardized inocula for quality control of culture media. These procedures apply in the general case but some microorganisms can require special conditions for preparation, e.g. anaerobes, moulds, halophilic, osmophilic or xerophilic microorganisms, and those with special growth or nutritional requirements.

### 5.4.2 Preparation

#### 5.4.2.1 Preparation of stock cultures

When required inoculate a solid culture medium e.g. Tryptone Soya agar (TSA) or Blood TSA, from reference stock in a way to achieve single colonies. Incubate under appropriate conditions, e.g. for most aerobic bacteria 18 h to 24 h at 37 °C. Verify and archive documents, including check on traceability to reference strain and relevant characteristics, if reference stock is obtained from outside source. Preparation of stock cultures can be necessary for some strains, e.g. for quantitative tests. Document all stages.

Inspect this solid stock culture for purity and store at a suitable temperature (18 °C to 25 °C or 2 °C to 8 °C depending on the microorganisms) for up to four weeks.<sup>[36]</sup> Freezing at a temperature below -70 °C enables extended storage (e.g. maximum 5 years). Storage life at a higher temperature (e.g. -20 °C) is more limited (e.g. 1 year)<sup>[36].</sup>

It is possible to culture on numbers of suitable solid medium to obtain a large volume of stock culture (multiple plates may be prepared). It is also possible to obtain a further stock culture from an existing stock culture in compliance with quality assurance measures (e.g. checking phenotypic characteristics) and documenting the passages (see [Figure B.2](#)).

#### 5.4.2.2 Preparation of working cultures

Prepare working cultures from the stock culture (or when required from the reference stock) as a pure stationary phase culture in a non-selective liquid culture medium. For most aerobic bacteria this is normally achieved by incubation for 18 h to 24 h.

The working culture can be prepared from a commercial reference material, RM or CRM, or be prepared by the laboratory. The concentration of the prepared suspension shall be stable and homogeneous during its period of use.<sup>[7][10][11][21][29][30]</sup>

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

Depending on the size of the colonies, take one to two colonies from the stock culture medium with an inoculation loop. The use of a 1 µl loop is recommended in order to avoid too heavy an inoculum.

Transfer the inoculum to a non-selective liquid culture medium, e.g. Tryptone Soya broth (TSB), and mix carefully using a vortex mixer.

Incubate under appropriate conditions and for an appropriate time (e.g. for most aerobic bacteria 18 h to 24 h at 37 °C).

Use this working culture for a specified time (e.g. for maximum three days at an appropriate temperature to prevent significant change according to the microorganism).

For preparation and storage of bacterial and fungal spores as working cultures, see References [10] [11] [24] [25] [30].

#### 5.4.2.3 Preparation of a standardized test suspension (inoculum) for the test

From the working culture, prepare serial dilutions in a suitable diluent (for example, quarter-strength Ringer's solution, peptone salt solution). Use the most suitable dilution step for the target level of microorganisms (cfu) in a specified volume or refer to the supplier.

For every test microorganism, the suitable dilution to use as a test suspension (inoculum) should be determined from previous tests conducted under strictly standardized conditions for all steps.

Use the test suspension (inoculum) within a specified time, for example, up to 2 h at room temperature or within 24 h if stored at  $(5 \pm 3) ^\circ\text{C}$ . [98] Longer storage periods can be acceptable if the stability of the stored culture has been verified.

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

For quantitative testing, the reference count shall be verified each time on the reference medium. For CRM (which is certified for the specific method), a verification is not needed every time.

See [Annex K](#) for more details.

#### 5.4.2.4 Volumes of inocula

Volumes of inocula used for quantitative performance testing shall reflect those used under test conditions for the relevant culture media.

For diluents and liquid culture media used for quantitative testing, the volume of the inoculum shall be in the same ratio as used in the relevant International Standard, usually 10 % of the culture medium under test.

#### 5.4.2.5 Inoculum

##### 5.4.2.5.1 Inoculum for productivity testing

##### 5.4.2.5.1.1 Quantitative testing

For the quantitative enumeration test, a reference count of approximately 100 cfu is necessary to achieve sufficient precision (see [Table 1](#)). A practicable range will be 80 cfu to 120 cfu per test, obtained on the reference agar, duly checked/controlled in parallel or beforehand. If more colonies are counted, the precision is increased. [Table 1](#) shows the 95 % confidence intervals associated with colony counts.

This can necessitate the use of more than one plate to reach a total of approximately 100 cfu for counting. Increasing the number of plates can also be necessary for some moulds and for spreading colonies to obtain countable colonies. Two plates are enough with approximately 50 target colonies on each. Three and four plates are necessary when about 35 and 25 target colonies, respectively, appear on each.

For membrane filters, see ISO 7704.

For quantitative tests of diluents and liquid transport media, an inoculum is needed of around 100 cfu in the volume spread on the plates. See [9.2.1.2](#) and [9.3.2.1](#).

**Table 1 — Approximate 95 % confidence intervals for numbers of colonies assuming agreement with Poisson distribution** [\[21\]](#)[\[26\]](#)

| Number of colonies counted | Limiting precision (to nearest percentage) % | Approximate 95 % confidence limits |
|----------------------------|----------------------------------------------|------------------------------------|
| 500                        | ±9                                           | 455 to 545                         |
| 400                        | ±10                                          | 360 to 440                         |
| 320                        | ±11                                          | 284 to 356                         |
| 200                        | ±14                                          | 172 to 228                         |
| 100                        | ±20                                          | 80 to 120                          |
| 80                         | ±22                                          | 62 to 98                           |
| 50                         | ±28                                          | 36 to 64                           |
| 30                         | ±37                                          | 19 to 41                           |
| 20                         | ±47                                          | 11 to 29                           |
| 16                         | ±50                                          | 8 to 24                            |
| 10                         | ±60                                          | 4 to 16                            |
| 6                          | ±83                                          | 1 to 11                            |

#### 5.4.2.5.1.2 Qualitative testing

The volume of suspension used for testing should contain

- $10^3$  to  $10^4$  cfu for qualitative tests of plate culture media,
- $\leq 100$  cfu for productivity tests of pre-enrichment and enrichment culture media,
- $10^4$  to  $10^6$  cfu for qualitative tests of solid transport media.
- $\geq 10^4$  cfu for qualitative tests of confirmation media and reagents

#### 5.4.2.5.2 Inoculum for selectivity testing

For selectivity testing of culture media, a suspension of the non-target microorganism containing at least  $10^4$  cfu is inoculated on to the plate or into the tube of culture medium.

Where the volume of liquid culture medium is greater than 10 ml, a proportionally greater inoculum of the non-target microorganism may be required. Please refer to [\[100\]](#) and [\[101\]](#) for guidance.

#### 5.4.2.5.3 Inoculum for specificity testing

For qualitative tests of plate culture media, for specificity an inoculum of at least  $10^3$  cfu is needed. Where a quantitative method is used to determine productivity, that plate can also be used to read the specificity of the medium.

#### 5.4.2.6 Incubation

Incubate the inoculated culture media in accordance with the conditions described in the relevant International Standard. [Annex E](#), [Table E.1](#) gives the incubation conditions to be used in specified International Standards for food microbiology and [Table E.2](#) gives the incubation conditions to be used in specified International Standards for water microbiology.

To avoid loss of moisture in agar culture media during incubation, see [4.6](#).

The shortest permissible incubation time in the International Standard concerned should be used for the target microorganism(s), while the longest permissible incubation time should be used when determining selectivity [9] [10].

## 6 Quality control and performance testing of culture media and reagents

### 6.1 General requirements

The following subclauses describe requirements for all culture media and reagents. They are applicable regardless of the batch size.

In practice, samples can contain stressed microorganisms. The suitability of the culture medium with respect to the recovery of stressed cfu should be taken into account [21] [31] [32] [33].

The quality of culture media and reagents depends on the quality of the basic ingredients, correct formulation, quality of preparation procedures, elimination of microbial contamination and appropriate packaging and storage conditions.

Adapt the quality control of the culture media and reagents to the use for which the culture media are intended (e.g. qualitative or quantitative). Before use, evaluate the performance of each batch of culture medium according to the culture media categories described in 6.4. If testing before use is not possible due to the lability of the culture medium or supplement, perform parallel performance testing alongside the sample testing. The performance of confirmation reagents shall be verified according to 6.6.2.

### 6.2 Physical and chemical quality control

Finished culture media shall comply with the physico-chemical characteristics as specified in the corresponding standards. Furthermore, quality assessment by visual inspection shall ensure that each culture medium conforms to stated recommendations, e.g.

- fill volume and/or thickness,
- appearance, colour and homogeneity,
- gel consistency, and
- moisture content,

In addition, pH value shall be determined. See Annex L.

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

### 6.3 Microbiological quality control

#### 6.3.1 General

Carry out the microbiological performance tests on representative items of the batch of end product of culture medium [6] [8] [9] [21].

#### 6.3.2 Reference culture medium

In order to ensure the reliability of results of performance testing, the reference culture medium used shall be of consistent good quality that meets a defined technical specification.

Examples of aspects to be considered by the user are the following:

- use of a quantitative RM (see 3.4.7) containing a well-defined number of microorganisms when evaluating a reference culture medium;

- use of a defined production process including remelting, if applicable;
- use of the same manufacturer/source for provision of the culture medium/ingredients;
- use of a larger range of test microorganisms when commissioning (to cover the range of microorganisms sought);
- the choice of “reference culture medium” for evaluation purposes;
- appropriate procedures for assuring the quality when in use as a reference culture medium.

It might not be necessary to include all of the above aspects when evaluating the suitability of the reference medium. The laboratory shall justify its choice of procedure.

Suitable test microorganisms, method of control and acceptance criteria for the reference culture media Tryptone Soya agar (TSA) and Sabouraud dextrose agar (SDA) are described in Annex N. Other non-selective reference culture media can be used if the above criteria are satisfied.

### 6.3.3 Microbial contamination

Test an appropriate quantity, depending on the size of the batch of culture medium, for the absence of microbial contamination (sterility) by incubation under appropriate conditions.

The samples to be tested shall be at least one plate or tube for small batches (<100 units). For larger batches, producers shall draw up specifications, e.g. based on culture media components, process parameters and limits and type of packaging using appropriate acceptable quality limits. Additional information is given in ISO 2859-1<sup>[6]</sup>, other national standards and other sources<sup>[9]</sup>.

Establish and justify acceptance criteria for each culture medium.

## 6.4 General guidance for microbiological performance testing

### 6.4.1 General

To evaluate a batch of complete culture medium, nutrient components or supplements, growth shall be appropriately assessed by either quantitative or qualitative methods as described in this International Standard.

Inoculate solid, semi-solid or liquid culture media with an appropriate volume (5.4.2.4) of the working culture of each of the defined test microorganisms using an appropriate device and following the inoculation technique described in relevant International Standards; see [Annex E](#).

Examples of quantitative and qualitative testing methods for solid culture media and liquid culture media are described in this International Standard. Any of the methods described can be chosen and not all methods need to be used.

When culture media are intended for enumeration purposes, quantitative testing methods shall be performed.

For testing combinations of solid culture media with membrane filters, use ISO 7704.

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

Suitable test microorganisms, methods of control and acceptance criteria are listed in [Annexes E](#) and the specific standards.

Guidance on the testing frequency and requirements to be carried out by the end user laboratories are described in [Table 2](#).

Upon any failure of the culture media - either on quality control performance tests or in-use monitoring - a return to the monitoring of each batch should be undertaken until reliability is re-established (9).

These principles also apply to those culture media and reagents used in International Standards methods that are not described in [Annex E](#), or not captured in international Standards; this includes proprietary culture media and reagents, or other methods.

## 6.4.2 Ready-to-use culture media

### 6.4.2.1 Prepared culture media purchased from ISO/IEC 17025 accredited manufacturers <sup>(90)</sup>

Accredited culture media manufacturers are those that hold ISO/IEC 17025 accreditation for QC testing of the culture media they produce. Laboratories shall assure themselves that such accreditation is held by checking the current scope of the manufacturer's accreditation. The scope of accreditation will specify the types of prepared culture media for which the manufacturer can issue endorsed reports or certificates.

The testing frequency and requirements to be carried out by the end user laboratories are described in [Table 2](#).

When the manufacturer releases a product, it shall be labelled with the product name, batch number, date manufactured and expiry date. The customer also needs to have access to information on:

- testing and sterility protocols including test methods, and
- results of quality control and performance testing (e.g. microorganisms, pH, recovery etc.) for each batch produced and expected results.

### 6.4.2.2 Prepared culture media purchased from other manufacturing quality assurance systems <sup>(90)</sup>

The testing frequency and requirements to be carried out by the end user laboratories are described in [Table 2](#).

Certification of the operations of a manufacturer to ISO 9001 does not demonstrate technical competence.

Accordingly, facilities purchasing culture media from suppliers certified to ISO 9001 only will be required to perform complete QC testing on all culture media. This includes performance, sterility, and physical testing parameters on every batch received.

### 6.4.2.3 Prepared culture media produced in-house for distribution to satellite laboratories

In general, all laboratories, including satellite sites receiving prepared culture media from a parent site not holding accreditation for culture media QC, should carry out full QC (physical, sterility, performance) on each batch of each culture medium made. Alternatively, laboratories preparing culture media for distribution to satellite sites are encouraged to seek accreditation for culture media QC.

It is recognised that under defined circumstances laboratories need to produce culture media in-house (e.g. specialised culture media used by reference laboratories) which will not be available for purchase from commercial accredited manufacturers. In this situation, satellite sites receiving specialised culture media from a non-accredited parent site will not be required to perform full QC provided the following criteria are met:

- the parent site carries out QC evaluation on each batch of each culture medium made. A copy of the culture media preparation details and QC results for each batch of culture medium produced shall be made available to the satellite site;
- the receiving satellite site shall demonstrate that the culture media has not been adversely affected by transit, storage and change in environmental conditions;
- the sites shall be part of the one organisation; and
- the culture media must not be sold or provided to other facilities outside the organisation. If any of the above is not met, the requirement for full QC at the satellite site applies.

#### 6.4.3 Culture media being prepared from commercially available dehydrated formulations

The testing frequency and requirements to be carried out by the end user laboratories are described in [Table 2](#).

For those culture media not described in [Annexes E](#), or not described in the individual International Standards pertaining to the Scope of this document, quality control can be specified according to the following recommendations:

- For those culture media which contain no indicators or selective agents, a restricted number of strains can be used.
- For those culture media which do contain indicators or selective agents, strains which demonstrate the function of the indicator(s) and selectivity should be utilized.
- For complex culture media, i.e. with added supplements, each batch should be verified with strains with characteristics listed in [5.2](#).

#### 6.4.4 Culture media being prepared from basic individual components

In addition to the requirements stated above, full quantitative testing shall be performed by the end user in order to monitor trends in quality of basic materials, productivity of the culture medium and in-house laboratory production protocols.

This includes performance, sterility, and physical testing parameters on every batch received.

The testing frequency and requirements to be carried out are described in [Table 2](#).

**Table 2 — Quality control and performance testing to be carried out by the end user laboratories according to type of medium**

| Type of media according to preparation method                                                                                                                                                                                                                                                                                               | Risk associated with type                                                                                                                                                                                                                                                                                                                                                         | Type of control                 | Manufacturer demonstrating QC competence <sup>(1)</sup> |                           | Other manufacturing QA systems |                           |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|---------------------------------------------------------|---------------------------|--------------------------------|---------------------------|
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   |                                 | New media or new manufacturer                           | Subsequent batches        | New media or new manufacturer  | Subsequent batches        |
| Ready-to-use culture media                                                                                                                                                                                                                                                                                                                  | Transport and storage conditions (temperature/time/light)                                                                                                                                                                                                                                                                                                                         | Delivery check (4.1.2)          | ●                                                       | ●                         | ●                              | ●                         |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Physical & chemical (6.2)       | –                                                       | –                         | ●                              | ●                         |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Microbial contamination (6.3.3) | –                                                       | –                         | ●                              | ●                         |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Performance testing             | ● <sup>(2)</sup>                                        | ● <sup>(3)</sup>          | ● <sup>(2)</sup>               | ●                         |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   |                                 |                                                         |                           |                                |                           |
| Ready-to-use culture media with melting and/or supplementing                                                                                                                                                                                                                                                                                | Transport and storage conditions (temperature/time/light)<br>Melting conditions (time/temperature)<br>Holding conditions (time/temperature)<br>Correct addition supplements (preparation/aseptic addition/final concentration)                                                                                                                                                    |                                 | New media or new manufacturer                           | Subsequent batches        | New media or new manufacturer  | Subsequent batches        |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Delivery check (4.1.2)          | ●                                                       | ●                         | ●                              | ●                         |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Physical & chemical (6.2)       | ● <sup>(4)</sup>                                        | –                         | ●                              | ●                         |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Microbial contamination (6.3.3) | ●                                                       | ●                         | ●                              | ●                         |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Performance testing             | ● <sup>(2)</sup>                                        | ● <sup>(5)</sup>          | ● <sup>(2)</sup>               | ●                         |
| Culture media prepared from commercial dehydrated formulations                                                                                                                                                                                                                                                                              | Transport and storage conditions DCM (temperature/time/light)<br>Preparation (concentration, pH, heat treatment)<br>Quality of the water<br>Storage prepared medium (time/temperature/light)                                                                                                                                                                                      |                                 | New media or new manufacturer                           | Each user lab preparation | New media or new manufacturer  | Each user lab preparation |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Delivery check (4.1.2)          | ●                                                       | ●                         | ●                              | ●                         |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Physical & chemical (6.2)       | ●                                                       | ●                         | ●                              | ●                         |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Microbial contamination (6.3.3) | ●                                                       | ●                         | ●                              | ●                         |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Performance testing             | ● <sup>(2)</sup>                                        | ● <sup>(6)</sup>          | ● <sup>(2)</sup>               | ●                         |
| Culture medium prepared from commercial dehydrated formulations with melting and/or supplementing                                                                                                                                                                                                                                           | Storage prepared medium (time/temperature/light)<br>Melting conditions (time/temperature)<br>Holding conditions (time/temperature)<br>Quality supplement(s) and water used to reconstitute supplement<br>Correct addition supplements (preparation/aseptic addition/final concentration)                                                                                          |                                 | New media or new manufacturer                           | Each user lab preparation | New media or new manufacturer  | Each user lab preparation |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Delivery check (4.1.2)          | ●                                                       | ●                         | ●                              | ●                         |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Physical & chemical (6.2)       | ● <sup>(4)</sup>                                        | –                         | ●                              | ●                         |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Microbial contamination (6.3.3) | –                                                       | –                         | ●                              | ●                         |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Performance testing             | ● <sup>(2)</sup>                                        | ● <sup>(7)</sup>          | ● <sup>(2)</sup>               | ●                         |
| Culture media prepared from basic individual components                                                                                                                                                                                                                                                                                     | Quality individual components<br>Storage conditions (temperature/time/light)<br>Preparation (concentration, pH, heat treatment)<br>Storage prepared medium (time/temperature/light)<br>Melting conditions (time/temperature)<br>Holding conditions (time/temperature)<br>Quality supplement(s)<br>Correct addition supplements (preparation/aseptic addition/final concentration) |                                 | For each user lab preparation                           |                           |                                |                           |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Delivery check (4.1.2)          |                                                         | ●                         |                                |                           |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Physical & chemical (6.2)       |                                                         | ●                         |                                |                           |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Microbial contamination (6.3.3) |                                                         | ●                         |                                |                           |
|                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   | Performance testing             |                                                         | ● <sup>(8)</sup>          |                                |                           |
| Key ● shall be completed – not required                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                   |                                 |                                                         |                           |                                |                           |
| <sup>(1)</sup> Competence may be demonstrated by ISO 17025 accreditation following ISO 11133                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                   |                                 |                                                         |                           |                                |                           |
| <sup>(2)</sup> Strains to be used as a minimum according to Annex E or the specific standard.                                                                                                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                   |                                 |                                                         |                           |                                |                           |
| <sup>(3)</sup> The testing frequency can be significantly reduced (e.g. once batch per year). Strains to be used as a minimum according to Annex E or the specific standard.                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                   |                                 |                                                         |                           |                                |                           |
| <sup>(4)</sup> pH and microbial contamination not required when only remelting.                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                   |                                 |                                                         |                           |                                |                           |
| <sup>(5)</sup> The testing frequency can be reduced but shall be greater than defined in footnote 3 (e.g. one out of every ten batches per year). Strains to be used as a minimum according to Annex E or the specific standard.                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                   |                                 |                                                         |                           |                                |                           |
| <sup>(6)</sup> The testing frequency can be reduced but shall be greater than defined in footnote 5 (e.g. one out every four batches per year). Strains to be used as a minimum according to the respective Annexes (e.g. Annex e, f or specific standards)                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                   |                                 |                                                         |                           |                                |                           |
| <sup>(7)</sup> Once the testing frequency of footnote 6 has been met, for subsequent aliquots (e.g. bottles/tubes of that batch) requiring melting and/or supplementing, the testing frequency can be again reduced (e.g. one of ten aliquot of that batch). Strains to be used as a minimum according to Annex E or the specific standard. |                                                                                                                                                                                                                                                                                                                                                                                   |                                 |                                                         |                           |                                |                           |
| <sup>(8)</sup> All strains according to Annex E or the specific standard.                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                   |                                 |                                                         |                           |                                |                           |

## 6.5 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.

If satisfactory performance is not achieved, see [Annex H](#) for possible reasons.

## 6.6 Confirmation culture media and reagents

### 6.6.1 Confirmation culture media

The performance of all culture media (in-house produced or ready to use) used for confirmation tests, and reagents, shall be verified before use. Appropriate positive and negative test microorganisms shall be used for verification in a similar way to that described in the specific International Standard<sup>[9][16]</sup>.

Suitable test microorganisms are described in [Annex E](#), or the specific standards, and prepared as described in [5.4](#).

For each media a minimum of one positive and one negative test microorganisms shall be tested. These tests can be performed in parallel to the confirmatory tests.

## 6.6.2 Confirmation reagents

Performance of Gram stain solutions, reagents, such as Kovacs, VP, nitrite, oxidase, catalase and other reagents used to demonstrate a biochemical characteristic, shall be verified before use. Use appropriate positive and negative strains for verification and establish a shelf-life. It is recommended that analytical grade reagents be used for confirmatory tests. If commercially prepared reagents are used, follow the manufacturer's instructions for storage, use and expiry<sup>[18][19]</sup>.

Suitable test microorganisms are described in [Annex E](#), or the specific standards, and prepared as described in [5.4](#).

## 7 Methods for performance testing of solid culture media

### 7.1 General

This clause describes quantitative and qualitative performance testing for solid culture media specified in food and water standards.

These are general methods suitable for most culture media. They might not be suitable for testing some types of culture media for recovery of moulds. Flow chart summaries of each method can be found in [Annex C](#).

### 7.2 Methods for quantitative tests

#### 7.2.1 Methods for quantitative tests — Definitions

##### 7.2.1.1 Productivity

Productivity shall reach a defined minimum limit (see corresponding specific International Standard or [Annex E](#)).

See [Annex G](#) for the use of control charts in monitoring the performance of solid culture media by the procedure described below.

For quantitative methods, the productivity ratio,  $P_R$ <sup>[21]</sup> is determined using [Formula \(1\)](#):

$$P_R = \frac{N_s}{N_o} \quad (1)$$

where

$N_s$  is the total count of colonies obtained on or in the culture medium under test, e.g. colony count on plates;

$N_o$  is the total count of colonies obtained on or in the defined reference culture medium, obtained from one or more plates, and shall be around 100 cfu; see [5.4.2.5.1](#).

For interpretation of results, see [7.2.2.3](#).

#### 7.2.2 Quantitative method for productivity of solid culture media

##### 7.2.2.1 General

This protocol requires the use of a quantified bacterial suspension (which can be a quantitative reference material/test suspension) with an appropriate concentration of a target strain. The recovery from the new batch of culture medium will be compared to the recovery from a non-selective culture medium (reference culture medium) or, in special cases, a previously accepted batch of the same culture media composition (e.g., GVPC Legionella medium). See also [Annex K](#).

### 7.2.2.2 Procedure

- a) Use working cultures and inocula of known appropriate concentration of a target strain and where appropriate also of non-target strain as described in [5.3.2](#), or suitable RM.
- b) Use one or more plates per microorganism. The number used will depend on the size of the batch, the confidence in the quality assurance production procedure and the reliability and number of microorganisms in the test suspension. The user laboratory shall justify the number used.
- c) Ensure that the surfaces of the plates are adequately dried; see [4.5.5](#).
- d) Inoculate by spreading the inoculum on the culture media or by the membrane filtration method to give counts that fall within the recommended limits given in [5.4.2.5.1](#) for quantitative testing.

The modified Miles-Misra surface drop method, other dropping systems or a spiral plater can also be used to give countable colonies on the plates.

- The pour plate method (or the above) should be used for culture media normally used for pour plate enumeration.
- Inoculate reference culture medium or plates from a previously accepted batch in the same way.
- Incubate the plates under the conditions defined in the individual International Standards. See also [5.4.2.6](#)
- Count the colonies present on each plate. Assess the size and appearance of the colonies on or in the culture medium under test by comparison with the recovery on a non-selective culture medium (reference culture medium) or a previously accepted batch of the same culture media composition.

### 7.2.2.3 Calculation and interpretation of results

For the quantitative enumeration test, around 100 cfu is necessary to achieve sufficient precision (see [Table 1](#)). This can necessitate the use of more than one plate per replicate.

The results will be accepted as valid if the following conditions are satisfied:

- each replicate shall contain a quantifiable number of target bacterial colonies;
- each single reported result is included in the standard range of analysis (up to 100 colonies for filtration methods and up to 150 colonies for surface methods). This also applies to a selective culture medium compared with a previous batch of the same medium e.g. *Legionella* plates

To interpret the results, calculate the productivity ratio,  $P_R$  (see [7.2.1.1](#)).

- the  $P_R$  shall be  $0,50 \leq P_R \leq 1,4$  for comparison of a selective culture medium with the non- selective reference culture medium.
- the  $P_R$  shall be  $0,70 \leq P_R \leq 1,4$  for comparison of a non-selective culture medium with a non- selective reference culture medium or as specified in the individual standard or method.
- the  $P_R$  shall be  $0,70 \leq P_R \leq 1,4$  for comparison of a reference culture medium with a reference culture medium or as specified in the standard or [Annex G](#).

If the  $P_R$  exceeds 1,4, identify the reason.

For special cases, see [Annexes E](#), and [J](#). These criteria might not be applicable to culture media which are not specified in [Annex E](#), or not described in the individual International Standards pertaining to the Scope of this document; e.g., those described in local standards, or for proprietary culture media.

Note that the  $P_R$  shown above is at  $\geq 0,50$  or  $\geq 0,70$  as a minimum. Laboratories may tighten their acceptance criteria if their results are consistently above the minimum.

#### 7.2.2.4 Using recovery from reference materials

This protocol uses reference materials (RMs), CRMs or internally produced RMs to provide a stable bacterial suspension containing a known number of colony-forming units of the target or non-target strain. The recovery from the new batch of culture medium will be compared to the expected number of cfu from the RM, CRM or internally produced RM.

Critical difference can be used for the calculation of tolerance limits (see ISO 5725-6<sup>[34]</sup>). See [Table 1](#).

For preparation and assessment of internal RMs, see References [\[21\]](#) and [\[29\]](#). For preparation and assessment of commercially supplied RMs, follow the manufacturer's instructions.

The quality of the RM shall be verified on the reference culture medium.

### 7.3 Testing of culture media used for membrane filtration

The quality of the membrane filters used shall be previously evaluated to demonstrate their suitability for use; see ISO 7704.

### 7.4 Testing of culture media used as contact plates

Culture media can be used as contact plates for surface sampling.

#### 7.4.1 Procedure

Use working cultures and inocula prepared as described in [5.4.2.1](#).

For quantitative testing of non-selective contact plates of a reduced diameter (usually 55 mm to 60 mm), an inoculum of 40 cfu to 80 cfu per test, with a minimum number of 10 cfu per plate, is used. The use of more than one plate per test will increase the precision. Where a medium contains neutralizers (e.g., polysorbate (tween), and/or lecithin), a slight inhibition effect may be observed compared to the reference medium (e.g., Tryptone Soya Agar (TSA)); a level of around 100 cfu is inoculated per test.

The spread plate technique, avoiding the rounded edges, shall be used ([7.2.2.2](#)).

The reference culture medium (usually a plate of 90 mm diameter), can be inoculated with the same number of cfu per test, to facilitate plating and interpretation of results.

It is strongly advised to not test the performance of contact plates onto surfaces, to avoid spread of microorganisms in the laboratory.

#### 7.4.2 Interpretation of results

For performance testing of non-selective contact plates, calculate the productivity ratio  $P_R$  in accordance with [7.2.1.1](#). Performance testing details for contact plates with added neutralizers are given in [Annex E](#).

### 7.5 Methods for qualitative tests for determination of productivity and selectivity

#### 7.5.1 Qualitative streaking method for determination of productivity and selectivity

##### 7.5.1.1 Procedure

Use working cultures and inocula as described in [5.4.2.5.1.2](#)

For productivity and specificity, use a plate of test culture medium and streak each test microorganism in a way to obtain discrete colonies.

For selectivity, use one plate of test culture medium and streak each test microorganism as a single straight line using a 1 µl loop on the surface of the test culture medium. Several test microorganisms can be streaked