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

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

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Foreword

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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

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 9, *Food products*, in collaboration with Technical Committee ISO/TC 147 *Water quality*, Subcommittee SC 4, *Microbiological methods*.

This first edition of ISO 11133 replaces the second edition of ISO/TS 11133-1 (ISO/TS 11133-1:2009) and the first edition of ISO/TS 11133-2:2003, which have been technically revised. It also incorporates the Amendment ISO/TS 11133-2:2003/Amd.1:2011. In particular, it also includes requirements for microbiology media for water testing. It supersedes ISO 9998:1991.

Introduction

In laboratories carrying out microbiological examinations, the main objectives are to maintain, resuscitate, grow, detect and/or enumerate a wide variety of microorganisms. Culture media are used in all traditional microbiological culture techniques and also for many alternative techniques. Many formulae of culture media are commercially available and many more, designed for specific growth purposes, are described in the literature.

Many tests and procedures depend upon culture media being capable of providing consistent and reproducible results. The requirements for media may be specific to both the sample and the organisms to be detected. Culture media meeting established performance criteria are therefore a pre-requisite for any reliable microbiological work. Sufficient testing should be carried out to demonstrate

a) the acceptability of each batch of medium,

b) that the medium is “fit for purpose”, and

c) that the medium can produce consistent results.

These three criteria are an essential part of internal quality control procedures and, with appropriate documentation, will permit effective monitoring of culture media and contribute to the production of both accurate and reliable data. For reliable microbiological analysis it is essential to use culture media of proven quality. For all media described in standard methods it is essential to define the minimum acceptance criteria required to 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.

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

In addition the acceptance criteria measured by the methods defined in this International Standard can be used by all microbiological laboratories to evaluate the productive, selective and/or elective properties of a culture medium.

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

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

1 Scope

This International Standard defines terms related to quality assurance of culture media and specifies the requirements for the preparation of culture media 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 intended for consumption or used in food production.

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

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

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

— non-commercial bodies supplying media to third parties;

— microbiological laboratories preparing culture media for their own use.

2 Normative references

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

ISO 6887-1, *Microbiology of food and animal feed — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 6887-2, *Microbiology of food and animal feed — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products*

ISO 6887-3, *Microbiology of food and animal feed — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products*

ISO 6887-4, *Microbiology of food and animal feed — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 4: Specific rules for the preparation of miscellaneous products*

ISO 6887-5, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 5: Specific rules for the preparation of milk and milk products*

ISO 6887-6, *Microbiology of food and animal feed — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 6: Specific rules for the preparation of samples taken at the primary production stage*

ISO 7704, *Water quality — Evaluation of membrane filters used for microbiological analyses*

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

ISO 8199, *Water quality — General guidance on the enumeration of micro-organisms by culture*

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.

NOTE 2 Tables E.2 and F.2 give explanations of media name abbreviated terms.

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
lot of culture medium
homogeneous and fully traceable unit of a medium referring to a defined amount of bulk, semi-finished product or end product, which is consistent in type and quality and which has been produced within one defined production period, having been assigned the same batch (or lot) number

3.1.3
chromogenic substrate
fluorogenic substrate
substrate containing a chromophore/fluorophore group and a substrate utilizable by bacteria or fungi

Note 1 to entry: After splitting the chromogenic/fluorogenic substrate, the chromophore/fluorophore is released and a coloured/fluorescent end product becomes visible/can be detected using an ultraviolet (UV) lamp.

3.2 Terminology of performance testing

3.2.1
performance of culture medium
response of a culture medium to challenge by test organisms under defined conditions

3.2.2
target microorganism
microorganism or group of microorganisms to be detected or enumerated

3.2.3
non-target microorganism
microorganism that is suppressed by the medium and/or conditions of incubation or does not show expected characteristics of the target microorganism

3.2.4
productivity of culture medium
level of 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 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

Note 1 to entry: When used in connection with compound words, this term is often shortened to read "medium" (e.g. enrichment medium).

3.3.2 Culture media classified by composition

3.3.2.1
chemically defined 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 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 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 organisms, 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 medium
culture medium consisting of an aqueous solution of one or more constituents, such as peptone water and nutrient broth

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 media in tubes, flasks or bottles are commonly called "broths".

3.3.3.2
solid medium
semi-solid medium
liquid medium containing solidifying substances (e.g. agar-agar, gelatin) in different concentrations

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

Note 2 to entry: Solid media poured into Petri dishes are commonly called “plates”. Solid media poured into tubes or small bottles that are kept in slanted positions while the media are solidifying are often called “slants” or “slopes”. If the 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 may 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 medium
medium enabling stressed and damaged microorganisms to repair and recover their capacity for normal growth without necessarily promoting their multiplication

EXAMPLE Buffered peptone water

Note 1 to entry: A resuscitation medium may also be used as a pre-enrichment medium, e.g. buffered peptone water.

3.3.4.5
pre-enrichment medium
enrichment medium
generally liquid medium which, due to its composition, provides particularly favourable conditions for multiplication of microorganisms

EXAMPLE Tryptone soya broth

3.3.4.5.1
selective enrichment medium
enrichment 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)

3.3.4.5.2
non-selective enrichment medium
enrichment medium that allows the growth of a wide variety of microorganisms

EXAMPLE Brain heart infusion broth

3.3.4.6
isolation medium
solid or semi-solid medium that allows the growth of microorganisms

3.3.4.6.1
selective isolation medium
isolation medium that allows growth of specific target microorganisms, while inhibiting, totally or partially, other microorganisms

EXAMPLE Modified charcoal cefoperazone deoxycholate agar (mCCD agar)

3.3.4.6.2
non-selective isolation medium
isolation 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 precise detection of target microorganisms

EXAMPLE TBX agar, MUG/EC medium

3.3.4.7
differential medium
characterization medium
medium that permits the testing of one or more physiological/biochemical characteristics of the microorganisms for their identification

EXAMPLE TBX agar, Lactose agar with tergitol 7 and TTC

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

3.3.4.8
identification medium
medium designed to produce a specific identification reaction which usually does not require any additional confirmatory test

EXAMPLE Bile aesculin azide agar

3.3.4.9
enumeration medium
selective or non-selective culture medium that enables a quantification of the microorganisms

EXAMPLE Baird-Parker agar, Yeast extract agar

Note 1 to entry: An enumeration medium may include the properties of a resuscitation and/or enrichment medium.

3.3.4.10
confirmation medium
medium that contributes to the identification or characterization of the microorganism following a preliminary resuscitation and/or enrichment and/or isolation step

EXAMPLE Kligler iron agar

3.3.4.11
medium containing neutralisers
transport medium, dilution medium or culture medium containing neutralizing ingredients to inactivate detergents/disinfectants or other biocidal agents

3.3.4.12
medium having multiple uses
medium assigned to several categories

EXAMPLE Blood agar is a resuscitation medium according to 3.3.4.4, an isolation medium according to 3.4.4.6 and a differential medium according to 3.3.4.7 used for detection of haemolysis. Buffered peptone water is a diluent according to 3.3.4.3 and a pre-enrichment medium according to 3.3.4.5.

3.3.4.13
reference medium
medium, usually non-selective, for comparative evaluation of performance independent of the medium under test and demonstrated to be suitable for control use

EXAMPLE Tryptone soya agar (TSA)

3.3.5 Culture media classified according to preparation method

3.3.5.1
ready-to-use medium
liquid, solid or semi-solid medium that is supplied in plates, bottles, tubes or other containers, in ready-to-use form or ready-to-use after remelting or ready-to-use after remelting and supplementing

3.3.5.1.1
finished culture medium
medium in a form that is ready for inoculation

3.3.5.1.2
ready-to-use medium after remelting
medium to be remelted, for instance for use in the pour-plate technique or to be poured into Petri dishes

3.3.5.1.3
ready-to-use medium after remelting and supplementing
medium to be remelted, supplemented and dispensed before use (incomplete ready-to-use medium)

EXAMPLE Tryptose sulphite cycloserine (TSC) agar, Baird- Parker or Rabbit Plasma Fibrinogen (RPF) agar

3.3.5.2
medium prepared from commercially dehydrated formulations
medium in dry form which requires rehydration and processing before use, resulting in one of two kinds of media:

— a complete medium;

— an incomplete medium to which supplements are added before use

EXAMPLE Powders, compacted granules, lyophilized products

3.3.5.3
medium prepared from individual components
medium produced by a microbiology laboratory entirely from its individual ingredients

3.4 Terminology for test microorganisms

3.4.1
test organism
microorganism generally used for performance testing of culture media

Note 1 to entry: Test organisms 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, i.e. a culture collection, which is a member of the World Federation of Culture Collections (WFCC) or the European Culture Collections' Organisation (ECCO), and defined to at least the genus and species level, 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 material
RM
material containing a quantity of revivable microorganisms, sufficiently homogenous and stable with respect to quantity of revivable microorganisms, which has been established to be fit for its intended use in a measurement process

Note 1 to entry: See Reference [3].

3.4.7
certified reference material
CRM
reference material characterized by a metrologically valid procedure for the quantity of revivable microorganisms, accompanied by a certificate that provides the value of the specified quantity of revivable microorganisms, its associated uncertainty and a statement of metrological traceability

Note 1 to entry: See Reference [3].

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 media to third parties):

— name of the medium, 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;

— batch number;

— target pH of the complete medium;

— storage information and expiry date;

— assigned shelf-life;

— quality control certificate showing test organisms 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), check the following:

— identification of the product;

— integrity of packaging;

— 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 media and supplements

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. Purchases should be planned 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.

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

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

4.3 Laboratory preparation of media

4.3.1 General

The accurate preparation of culture media 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 media and other components, particularly those containing hazardous materials i.e. bile salts, sodium azide, antibiotics or other selective agents.

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

For media prepared from individual components, follow the formulation precisely. 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 medium components

Formulation of basic media components is described in the specific International Standards (see the Bibliography). When available, the molecular mass and the CAS[1)] 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 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, 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.

The purified water shall be stored in tightly closed containers made from an inert material (neutral glass, polyethylene, etc.) which shall be 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 °C ± 1 °C for 68 h ± 4 h or using an equivalent method.

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 μScm^{-1} (equivalent to a resistivity ≥ 0,04 MΩ cm) and preferably below 5 μScm^{-1} (grade 3 water, see ISO 3696[5]) at 25 °C, unless otherwise required by design. The conductivity of the water should be checked before use.

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.

4.3.4 Weighing and rehydration

Following the appropriate safety precautions, carefully weigh the required amount of dehydrated medium or individual ingredients and progressively mix with the required amount of water to avoid formation of lumps. Use a balance of sufficient discrimination; the maximum permissible errors should be 1 % or better, 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 media need rapid dispersion by instant and repeated or continuous stirring followed by heating, if necessary, to dissolve. Media containing agar should be allowed to soak for several minutes before heating with mixing to dissolve and then dispensing if necessary before autoclaving. Avoid over-heating the medium.

4.3.6 Measurement and adjustment of pH

Measure the pH using a pH meter and adjust before sterilization if necessary, so that after sterilizing and cooling to 25 °C the medium is at the required pH ± 0,2 pH units, unless otherwise stated. The adjustment is normally carried out using a sodium hydroxide solution of approximately 40 g/l [c (NaOH) = 1 mol/l] or dilute hydrochloric acid of approximately 36,5 g/l [c (HCl) about 1 mol/l]. If adjustment is performed after sterilization, use a sterilized solution. Additional information on pH measurement is given in ISO 7218 and ISO 8199.

NOTE Commercially manufactured media can show significant changes in pH before and after autoclaving. However, provided good quality distilled or deionized water is used, pH adjustments before autoclaving are usually not necessary.

4.3.7 Dispensing

Dispense the 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 media do not need autoclaving but can be used following boiling. For example media for *Enterobacteriaceae* containing brilliant green are particularly sensitive to heat and light and should be rapidly cooled after boiling and protected from strong light. Some reagents can be used without sterilization. In all cases, make reference to appropriate the 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 media preparator.

For containers with volumes of media greater than 1 000 ml, 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.

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

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

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

Sterilization by heat should be evaluated using F_0 values, taking into account the heat treatment during heating and cooling. The heat treatment should be defined for the particular load to be treated to ensure suitable treatment for containers irrespective of placement in the autoclave.

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 7218 or 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, the user should choose a suitable membrane type, e.g. low protein-binding membrane, and pre-wet the filter.

4.3.8.4 Preparation of supplements

CAUTION — Supplements containing toxic agents, particularly antibiotics, shall be handled with care avoiding dispersion of powder, which may 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 which, for antibiotic working solutions, is generally the same day. Under certain circumstances, antibiotic solutions may be stored frozen in suitable aliquots but should not be re-frozen after thawing. The potential loss of activity due to freezing shall be established with the manufacturer or tested by the user.

4.4 Storage and shelf-life of prepared media

4.4.1 Commercially supplied media

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

4.4.2 Laboratory prepared media

4.4.2.1 General

Identify all media in a way that ensures traceability.

The shelf-life of different media 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 media under conditions which prevent any modification 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 media is given in ISO 8199, Reference [17] and Reference [21].

It is recommended that media to which labile supplements have been added should be used on the day of preparation, unless otherwise specified in specific standards or results of laboratory shelf-life evaluation indicate a longer shelf-life is suitable (4.4.2.2). Solid 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

The expiry date for stored media shall be established by checking 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 media.

4.4.2.3 Storage of media in Petri dishes

Use the solidified medium immediately or store inverted under conditions which prevent deterioration and dehydration, i.e. in the dark and/or in the refrigerator at 5 °C ± 3 °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 may 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 cool 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). Media that have previously been autoclaved should be reheated for a minimum time to maintain 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. Caps of containers should be loosened before heating and tightened after removal from heat.

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

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

NOTE Media used in pour plate methods, which are added to the sample, are tempered to 44 °C to 47 °C, or as specified in the International Standard. Use a water bath set at 44 °C to 47 °C. Additional information on the use and verification of water baths is given in ISO 7218.

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

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

4.5.4 Preparation of solid 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 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 may be required. Allow the agar to cool and solidify by placing the plates with lids in place on a cool, horizontal surface.

Commercially prepared ready-to-use agar plates should be stored and used according to the manufacturer's instructions.

4.5.5 Preparation of plated 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 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 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 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 may 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 lids. 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 media in Petri dishes

During incubation, agar media will lose moisture. This can affect the growth of microorganisms in some circumstances. Factors influencing water loss are medium composition, amount of medium in the plates, the type of incubator i.e. fan-assisted or otherwise, humidity of the atmosphere in the incubator, the position and number of the plates in the incubator and the incubation temperature. 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 may be increased by placing an open container of water in the bottom. The water should be changed and containers disinfected frequently to avoid fungal contamination.

4.7 Disposal of media

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

5 Test organisms for performance testing

5.1 General

New or revised standards shall specify performance testing of culture media, including the specification of control strains and acceptance criteria, according to the requirements of Annex J.

5.2 Selection of test organisms

A set of test organisms 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 medium. The test organisms should primarily comprise strains that are widely available in reference culture collections, but well-characterized strains isolated by the laboratory may also be included. It is preferable to use strains which have originated from foods or water, although not all culture collections provide such information on strain origin.

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 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 medium may include:

— robust positive strains with typical characteristics of the target organism;

— weakly positive strains;

— negative strains not showing expected characteristics of the target organism (negative characteristics);

— partly or completely inhibited strains.

Annex E gives the test microorganisms to be used in specified food microbiology International Standards, and Annex F gives the test microorganisms to be used in specified water microbiology International Standards.

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.

5.3 Preservation and maintenance of test organisms

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 °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]

The number of transfers of test organisms should be documented to prevent excessive subculturing that increases the risk of phenotypic alteration. One passage is defined as the transfer from a viable culture to a fresh medium with growth of the microorganisms. Any form of subculturing 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 9001[2] 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 should ascertain whether the strain supplied is a reference strain or reference stock and how many passages have taken place before receipt and document the information.

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

5.3.3 Laboratory prepared reference stocks

Reference stock cultures prepared from reference strains (see Figure B.1) for performance testing purposes shall be maintained and handled in a manner that minimizes the opportunity for cross-contamination, mutation or alteration of typical characteristics. Reference stocks should be stored in multiple portions, usually either deep-frozen, e.g. below –70 °C, or lyophilized. At a higher temperature, duration of viability might be reduced and genetic modification might occur.

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

Reference stocks shall not be used 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). Aliquots shall be handled in a manner that avoids possible cross-contamination of the reference stock and/or its deterioration. Stock cultures should be prepared by re-suspending a portion of the reference stock in or on a non-selective growth medium; incubate to yield a stationary phase culture.

See 5.3.3 for storage and documentation requirements.

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

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

5.3.5 Working cultures

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

Working cultures shall not be used 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 listed in Annexes E and F.

The volumes of inocula and numbers of organisms 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 media. These procedures apply in the general case but some organisms can require special conditions for preparation, e.g. anaerobes, moulds, halophilic, osmophilic or xerophilic organisms, 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 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.

Inspect this solid stock culture for purity and use it for a specified time (e.g. for 14 days at an appropriate temperature to prevent significant change according to the organism).

5.4.2.2 Preparation of working cultures

Working cultures shall be prepared from the reference stock (or when required the stock culture) as a pure stationary phase culture in a non-selective broth. 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.[7][10][11][21][29][30]

Different techniques may 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 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 organism).

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 suspensions (inocula) for the test

Prepare serial dilutions in a suitable diluent (e.g. quarter-strength Ringer's solution, peptone salt solution) and use the most suitable dilution step for the desired number of organisms (cfu) in a specified volume.

The suitable dilution to use as a test inoculum should be determined from previous tests conducted under strictly standardized conditions for all steps.

Use the suspensions (inocula) within a specified time (e.g. up to 2 h at room temperature or within 24 h if stored at 5 °C ± 3 °C; longer storage periods may be acceptable if validated[10][21]).

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

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 media.

For diluents and liquid 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 medium under test.

5.4.2.5 Inoculum level

5.4.2.5.1 Inoculum level for productivity testing

5.4.2.5.1.1 Quantitative testing

For the quantitative enumeration test, a level of around 10^2 cfu is necessary to achieve sufficient precision (see Table 1). This may necessitate the use of more than one plate replicate.

A practicable range of 80 cfu to 120 cfu per plate with a minimum number of 50 cfu per plate should be used. For filters, the same number of cfu are needed using one or more filters. Table 1 shows the 95 % confidence intervals associated with colony counts.

For quantitative tests of diluents and liquid transport media, an inoculum level of 10^3 to 10^4 cfu is needed to achieve an inoculum of around 100 cfu in the volume spread on the plates.

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 used for testing should contain

— 10^3 to 10^4 cfu for qualitative tests of plate media,

— ≤ 100 cfu for productivity tests of pre-enrichment and enrichment media,

— 10^4 to 10^6 cfu for qualitative tests of solid transport media.

5.4.2.5.2 Inoculum level for selectivity testing

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

5.4.2.5.3 Inoculum level for specificity testing

For qualitative tests of plate media, for specificity an inoculum level of 10^3 to 10^4 cfu is needed.

5.4.2.6 Incubation

Incubate the inoculated culture media in accordance with the conditions described in the relevant International Standard. Annex E gives the incubation conditions to be used in specified International Standards for food microbiology and Annex F gives the incubation conditions to be used in specified International Standards for water microbiology.

To avoid loss of moisture in agar media during incubation, see 4.6.

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

6 Quality control and performance testing of culture media

6.1 General requirements

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

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

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

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

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.

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

The microbiological performance tests shall be carried out on a sample which is representative of a batch of end product.[6][8][9][21]

6.3.2 Reference medium

In order to ensure the reliability of results of performance testing, the reference medium used shall be of consistent high quality.

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

— use of a quantitative RM (see 3.4.6) containing a well-defined number of organisms when evaluating a reference medium;

— use of a defined production process including remelting, if applicable;

— use of the same manufacturer/source for provision of the medium/ingredients;

— use of a larger range of test organisms when commissioning (to cover the range of organisms sought);

— the choice of "reference medium" for evaluation purposes;

— appropriate procedures for assuring the quality when in use as a reference 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 organisms, method of control and acceptance criteria for the reference medium Tryptone Soya agar (TSA) are described in Annexes E and F. Other non-selective reference media may be used if the above criteria are satisfied.

6.3.3 Microbial contamination

An appropriate quantity, depending on the size of the batch of culture medium, shall be tested for 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 media components, process parameters and limits and type of packaging using appropriate acceptable quality limits. Additional information is given in ISO 2859-1,[6] other national standards and other sources.[9][21]

Acceptance criteria shall be established and justified for each medium.

6.4 General requirements 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.

Solid, semi-solid or liquid culture media shall be inoculated 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 Annexes E and F.

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

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

When a new medium or a new manufacturer is being evaluated, quantitative testing methods are recommended to provide additional information to support the change.

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

For testing combinations of solid 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 F.

The testing frequency shall be justified by the end user, taking into account the extent of preparation in the end user's laboratory and the level of quality assurance in place.

6.4.2 Ready-to-use media

Manufacturers of commercially available ready-to-use media, especially if according to ISO 9001, will have a quality programme in place and might issue a quality certificate with the media they supply. Under those conditions, the user might not need to carry out extensive testing on such media, but shall ensure that storage conditions are maintained as recommended by the manufacturers.

For ready-to-use finished media to which supplements have been added, and which have been controlled by the manufacturer in accordance with this International Standard, at least a qualitative test is recommended.

The user shall ensure that manufacturers of commercially available ready-to-use media have a quality programme in place for this range of products and issue quality control certificates meeting the requirements of this International Standard, specifying the expected and obtained results. The user laboratory shall also check documentary evidence to ensure that the manufacturers' acceptance criteria for performance testing satisfy their own internal requirements.

Periodic checks shall be carried out in order to demonstrate that the quality of media has been maintained during transport.

Checks shall also be performed following storage and further handling in the user's laboratory, e.g. melting of solid media. The frequency shall be justified.

For incomplete media to which supplements are added by the user laboratory (see 3.3.5.1), an additional check should be carried out either by checking the production records or by performing a qualitative test to ensure that the correct supplement has been added.

6.4.3 Media prepared from commercially available dehydrated formulations

For enumeration media, quantitative testing shall be performed. For other media, qualitative testing may be sufficient. Quantitative tests will give greater assurance of media quality.

For those media not described in Annexes E and F, quality control should be specified according to the following recommendations.

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

6.4.4 Media prepared from basic individual components

In addition to the requirements stated in 6.4.3, quantitative testing shall be performed in order to monitor trends in quality of basic materials, productivity of the medium and in-house laboratory production protocols.

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 media and reagents

6.6.1 Confirmation media

The performance of culture media used for confirmation tests shall be verified before use. Appropriate positive and negative test organisms shall be used for verification in a similar way to that described in the specific International Standard.[9][16]

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. Appropriate positive and negative strains shall be used for verification and a shelf-life should be established. 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 and use.[18][19]

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 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 Annexes E and F).

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[21] 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 7.2.2.1.2.

7.2.1.2 Selectivity

Selective culture media and a non-selective reference medium are inoculated with different dilutions of non-target organism(s).

The selectivity factor, S_F,[22] is calculated as given by Formula (2):

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

where

D_O is the highest dilution showing growth on the non-selective reference medium;

D_S is the highest dilution showing comparable growth on the selective test medium;

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

NOTE For example if D_o 10^{-4} = $\log_{10}$ 4,0 and D_S 10^{-3} = $\log_{10}$ 3,0 then the selectivity factor S_F = 1,0.

For interpretation of results, see 7.2.2.1.2.

7.2.2 Quantitative method for solid culture media

7.2.2.1 General

This protocol requires the use of a quantified bacterial suspension (which may 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 medium) or, in special cases, a previously accepted batch of the same media composition.

7.2.2.1.1 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) One or more plates per organism should be used. The number used will depend on the size of the batch, the confidence in the quality assurance production procedure and the reliability and level of the organism 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 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 may also be used to give countable colonies on the plates.

— The pour plate method shall be used for culture media normally used for enumeration in this way.

— Inoculate reference medium or plates from a previously accepted batch in the same way.

— Incubate the plates under the conditions defined in the individual International Standards.

— Count the colonies present on each plate. Assess the size and appearance of the colonies on or in the medium under test by comparison with the recovery on a non-selective culture medium (reference medium) or a previously accepted batch of the same media composition.

7.2.2.1.2 Calculation and interpretation of results

For the quantitative enumeration test, a level of around 100 cfu is necessary to achieve sufficient precision (see Table 1). This may 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 give a positive quantitative result (target bacterial growth);

— 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).

To interpret the results, calculate the productivity ratio, P_R (see 7.2.1.1), and where appropriate, the selectivity factor, S_F (see 7.2.1.2).

a) The P_R shall be ≥ 0,50 for comparison of a selective medium with the non-selective reference medium specified in Annexes E and F. The P_R shall be ≥ 0,70 for comparison of a non-selective medium with a non-selective reference medium or as specified in the standard or Annexes E and F. This also applies to special cases where comparison is made with the previous batch.

b) If the P_R exceeds 1,4 identify the reason.

c) The S_F of non-target microorganisms is at least 2.

For special cases, see Annexes E, F and J. These criteria might not be applicable to media which are not specified in Annexes E and F, e.g. those described in local standards.

7.2.2.2 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 unwanted 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 may be used for the calculation of tolerance limits (see ISO 5725-6[34]). See Table 1.

For preparation and assessment of internal RMs, see References [21] and [29].

The quality of the RM shall be verified on the reference 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; use ISO 7704.

To test the performance of a culture medium for use in membrane filtration, use working cultures and inocula as described in 5.4.2. Inoculate the suspension medium e.g. dilution fluid, sterile water, with a suitable inoculum level given in 5.4.2.5.

Filter the liquid according to the requirements of the specific International Standard. Place the membrane on the surface of the agar under test. Inoculate sufficient membranes/plates to obtain a total of approximately 100 cfu for productivity testing. Repeat with a new membrane and place the second membrane on the surface of the reference medium, using dilutions if required for selectivity testing. Incubate the plates according to the specific standard.

Repeat the process each time the batch of membranes changes as well as each new batch of medium.

If necessary, to evaluate the influence of the membrane on the result also spread the test inoculum on to the test medium and reference medium without the membranes.

7.4 Methods for qualitative tests

7.4.1 Qualitative streaking method

7.4.1.1 Procedure

Use working cultures and inocula as described in 5.4.2.

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

For selectivity, use one plate of test medium and streak each test microorganism as a single straight line using a 1 µl loop on the surface of the test medium. Several test microorganisms can be streaked on the same plate as parallel lines without crossing; streaks should be distinguishable to allow observation of typical morphology. Other standardized streaking methods can be used.

Incubate the plates under the conditions defined in the specific International Standard.

7.4.1.2 Interpretation of results

The amount of growth on the plates after incubation is assessed as follows:

— 0 corresponds to no growth;

— 1 corresponds to weak growth (either reduction in amount of growth or colony size);

— 2 corresponds to good growth.

Target microorganisms shall score 2 and have typical appearance, size and colony morphology. For selectivity tests, the degree of inhibition depends on the type of medium. The growth of non-target microorganisms shall be partly or completely inhibited.

7.4.2 Determination of specificity

A definition of specificity is given in 3.2.6. The specificity of the culture medium is given by essential indicative physiological characteristics to differentiate related organisms by the presence, absence and/or grade of expression of biochemical responses and colony sizes and morphology. For working culture and inocula requirements, see 5.4.2.

7.4.3 Other qualitative methods for solid media

Other qualitative methods may be used.[9][21]

8 Methods for performance testing of liquid culture media

8.1 General

This clause describes quantitative and qualitative methods for performance testing of liquid culture media. Flow chart summaries of each method can be found in Annex C.

8.2 Quantitative tube method for performance testing of liquid enrichment media (dilution to extinction method)

8.2.1 General

This method is a general method that may be used for non-selective or selective liquid media. It is also suitable for performance testing of liquid media used for enumeration, e.g. in most probable number methods.

8.2.2 Preparation of the dilution series

— Select a representative number of tubes; see 6.3.1.

— Prepare a suitable dilution series from the working culture of the target or non-target organism in a suitable diluent as described in ISO 6887-1 and ISO 8199 so as to achieve absence of organisms in

the highest dilution (extinction), e.g. from 10^{-1} to 10^{-10}. A decimal dilution series is most commonly used, but 1/5 or 1/2 dilution steps are also suitable.

— Use the dilution series within a specified time; see 5.4.2.3.

— At the time of use, transfer a known volume, e.g. 0,1 ml of each dilution to the surface of a non-selective agar plate and spread.

— Incubate under appropriate conditions for the organism concerned.

— Count the number of colonies on the agar plates at the lowest dilution containing up to 150 colonies and the number of colonies on higher dilutions than this and record.

8.2.3 Procedure for testing the liquid medium

— Select the same number of tubes of medium under test to correspond to the number of tubes in the dilution series.

— Using the dilutions prepared according to 8.2.2 and starting with the highest dilution, inoculate a known volume of the test organism suspension, e.g. 0.1 ml into the corresponding tube of medium.

— Incubate the tubes under the conditions described in the relevant International Standard; see 5.4.2.6.

— After incubation, use a separate 10 µl loop for each tube of incubated medium to subculture to a non-selective agar medium.

— Incubate the inoculated plates under conditions appropriate for the organism.

— After incubation, examine each plate for the presence or absence of growth.

NOTE For the target organism, it is usually sufficient to use the 10^{-5} to 10^{-8} dilutions. For non-target organisms, it is usually sufficient to use the 10^{-1} to 10^{-4} dilutions.

8.2.4 Calculation and interpretation of results

Productivity of the liquid enrichment medium is satisfactory if good growth (at least 10 cfu from a 10 µl loopful) of the target microorganism is obtained from the dilution producing fewer than 100 cfu (in 0.1 ml) on the plate.

For selective liquid media, determine the selectivity factor, S_F, from the highest dilution of the working culture showing good growth (at least 10 cfu) on the agar plate and the highest dilution of the inoculated selective liquid medium showing no growth (or less than 10 cfu) of the non-target microorganism on the non-selective agar plate. The S_F should be at least 2.

NOTE Additional methods for quantitative testing of liquid media for use in the evaluation of media under development or in comparative studies are described in Annex I.

8.3 Qualitative tube method for performance testing of selective liquid media

8.3.1 General

This method uses target, non-target, or a mixture of target and non-target organisms in the same tube.

8.3.2 Procedure

— Select a number of tubes each containing 10 ml of medium or 10 ml portions from each batch to be tested (see 3.1.2 and 6.3.1). Proceed as described below according to the requirements specified in Annexes E and F.

— Preparation of inocula: see 5.4.2.3.

— **Inoculation of target organisms**: Inoculate one tube of test broth with an inoculum containing ≤ 100 cfu of target microorganism and mix.

— **Inoculation of non-target microorganisms:** Inoculate one tube of test broth per microorganism with an inoculum containing a higher number (>1 000 cfu) and mix.

— **Inoculation of target and non-target organisms in the same tube** when required in Annexes E or F or when a new medium or new manufacturer is being evaluated. Inoculate one tube of test broth with ≤ 100 cells of target microorganism and the same tube with a higher number of non-target microorganisms (≥1 000 cells for every tube) and mix.

— Incubate the tubes under the conditions defined in the individual International Standards; see 5.4.2.6.

— Remove one loopful (10 µl) from the tube containing the target organism and streak on a plate of a non-selective medium (e.g. TSA).

— If a mixed culture of target and non-target organisms has been used, remove one loopful (10 µl) and streak on a plate of the specific medium for the target microorganism.

— Remove one loop (10 µl) from the culture of non-target microorganism and streak on a plate of a selective medium (e.g. XLD).

— Incubate the plates under the conditions defined in the individual International Standards.

If a larger volume of medium is used the user may choose whether to adjust the inoculum size proportionately in order to achieve equivalent results.

8.3.3 Calculation and interpretation of results

Productivity of the liquid test broth is satisfactory if good growth (at least 10 cfu or a line of confluent growth) of the target microorganism is obtained on the specific medium for that organism.

Selectivity of the liquid test broth is satisfactory if no growth (or less than 10 cfu) of non-target microorganisms occurs on the non-selective agar plate.

8.4 Qualitative single tube method (turbidity) for performance testing of liquid media

8.4.1 General

The method is suitable for performance testing of non-selective liquid culture media and selective media used for confirmation testing, e.g. Brilliant green bile lactose (BGBLB) broth.[41] The method is only qualitative and scores are therefore only indicative. Inherently turbid media can only be tested by this method if subcultured to a solid medium to demonstrate growth.

For clear media, the following notation is used:

— 0 equals no turbidity;

— 1 equals slight turbidity;

— 2 equals good turbidity.

8.4.2 Procedure

8.4.2.1 Pre-enrichment media

— Select a number of tubes each containing 10 ml of medium or 10 ml portions from each batch to be tested (see 3.1.2 and 6.3.1).

— For performance testing of pre-enrichment media, e.g. buffered peptone water (BPW), inoculate the medium with an appropriate inoculum volume (see specific International Standard) containing ≤ 100 cfu directly into the medium under test.

— Preparation of inocula: see 5.4.2.3.

— Incubate the tube under the conditions defined in the specific International Standard; see 5.4.2.6.

— Examine the medium for growth.

8.4.2.2 Confirmation media

— For performance testing of liquid confirmation media inoculate the medium under test with the working culture suspension (containing > 10^6 cfu/ml) using a 1 µl loop.

— Incubate the tube under the conditions defined in the individual International Standards; see 5.4.2.6.

— If the uninoculated medium is inherently turbid subculture to a solid medium, incubate the plates under the conditions defined in the individual standards and examine for growth.

8.4.3 Interpretation of results

— Qualitative evaluation shall be carried out visually by looking for good turbidity (i.e. 2) representing good growth; see 8.4.1. Qualitative evaluation of opaque media when produced is indicated by the presence of growth on the solid medium.

NOTE 1 Sometimes the growth of microorganisms can only be observed as a cell aggregation/deposit at the base of the tube or bottle. In this case, careful shaking can improve assessment and interpretation.

NOTE 2 Other characteristics, such as gas formation and colour change, can also be assessed by this method.

9 Methods for performance testing of diluents and transport media

9.1 General

Microbiological performance tests shall be carried out on a sample which is representative of the batch of end product; see 6.3.1.

9.2 Method for testing diluents

9.2.1 Method for quantitative testing of diluents

9.2.1.1 General

The method determines the ability of the diluent to support the survival of microorganisms without undue multiplication or reduction during the period of contact before plating on to agar or inoculation into liquid media.

9.2.1.2 Procedure

Inoculate a test portion (e.g. 9 ml) of the diluent with 1 ml of the test microorganism suspension containing around 10^4 cfu/ml and mix; for preparation of the inoculum see 5.4.2. Immediately remove 0,1 ml of inoculated diluent and spread over the surface of a non-selective agar (reference medium) such as TSA (t_0).

Hold the inoculated diluent at ambient temperature for the time lapse specified in the appropriate part of ISO 6887 (all parts) or ISO 8199 between the end of preparation of the initial suspension and the

moment when the inoculum comes into contact with the culture medium (usually 45 min). Mix and then remove the same volume (0,1 ml) and plate again on the reference medium (t_1).

Incubate the reference medium at an appropriate temperature and time e.g. 30 °C/72 h.

9.2.1.3 Reading and interpretation of results

After incubation count the colonies on the plates t_0 and t_1.

The number of microorganisms, t_1, after incubation of the diluent shall be within ± 30 % of the initial count (t_0).

9.3 Method for testing transport media

9.3.1 General

The method determines the ability of the transport medium to sustain the viability of the microorganism inoculated into it during the period of transport without undue multiplication or reduction of the level inoculated.

If transport systems are fitted with a sampling device, this shall be used for the inoculation of the transport medium. Otherwise, the inoculation shall be carried out under conditions which correspond to those occurring in practice.

Incubate the inoculated transport medium at an appropriate temperature and time according to usage in practice or given in the specified International Standard.[12][13]

EXAMPLE Transport systems for sample transport under cooled conditions are tested at a temperature of 5 °C ± 3 °C for the normal transport duration, e.g. for 24 h before repeating the plating out.

9.3.2 Method for quantitative testing of liquid transport media

9.3.2.1 Procedure

Inoculate a test portion (e.g. 10 ml) of the liquid transport medium with the appropriate test microorganism for which it will be used. Use an inoculum level of 10^3 to 10^4 cells into each 10 ml tube; for preparation of the inoculum (see 5.4.2). Immediately withdraw 0,1 ml of inoculated medium and spread over the surface of a non-selective agar medium (reference medium), such as TSA (t_0).

For the inoculation of transport systems with sampling devices, place the sampling device in an appropriate volume (e.g. 0,1 ml for swabs) of the dilution of working culture (containing 10^3 to 10^4 cells) for around 10 s and then inoculate the transport medium with the sampling device. Immediately withdraw 0,1 ml of inoculated liquid transport medium and spread over the surface of a non-selective agar medium (reference medium), such as TSA (t_0).

Incubate the inoculated transport medium and the reference medium at an appropriate temperature and time according to usage in practice or given in the specified International Standard, e.g. 25 °C/5 d for the transport medium and 30 °C/3 d for the reference medium, then repeat the plating out on the reference medium (t_1).

9.3.2.2 Reading and interpretation of results

After incubation count the colonies on the plates t_0 and t_1.

The number of microorganisms, t_1, after incubation of the transport medium shall be within ± 30 % of the initial count (t_0).

9.3.3 Method for qualitative testing of solid transport media

9.3.3.1 Procedure

Inoculate a test portion of the solid transport medium with the appropriate test microorganism for which it will be used. Use an inoculum level of 10^4 to 10^6 cells; for preparation of the inoculum (see 5.4.2).

For the inoculation of transport systems with sampling devices, place the sampling device in an appropriate volume (e.g. 100 µl for swabs) of the dilution of working culture (containing 10^4 to 10^6 cells) for around 10 s and then inoculate the transport medium with the sampling device.

Incubate the inoculated transport medium at an appropriate temperature and time according to usage in practice or given in the specified International Standard. Subculture on to a non-selective agar medium (reference medium), such as TSA and incubate according to usage in practice or given in the specified standard.

9.3.3.2 Reading and interpretation of results

After incubation, examine for the presence of growth on the non-selective agar.

There shall be visible growth of the microorganisms after incubation.

10 Documentation of test results

10.1 Information provided by the manufacturer

The manufacturer or supplier of the culture media shall provide, on request, the specific microbiological growth characteristics and general information relating to the specific batch of culture medium.

10.2 Traceability

All the data from routine performance testing should be documented in an appropriate way and kept for a sufficient period of time according to the quality system in use. The use of control sheets for documenting and evaluating the results of the tests is recommended (see Annex D).

Annex A
(informative)

Designation of the components of culture media in International Standards on microbiological analysis of food, animal feed and water

A.1 General

In order to harmonize the description of the various components of culture media in microbiological International Standard methods, ISO/TC 34/SC 9 have agreed the following designations.

A.2 Peptones

— Enzymatic digest of casein;

NOTE 1 This includes pancreatic or peptic digest of casein, tryptic digest of casein and tryptone.

— enzymatic digest of soy or soybean meal;

— enzymatic digest of animal tissues;

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

— enzymatic digest of heart;

— enzymatic digest of gelatin(e);

— enzymatic digest of animal and plant tissue;

NOTE 3 This includes tryptose.

— acid hydrolysis of casein.

A.3 Extracts and infusions

— Meat extract and meat infusion;

— brain-heart extract and brain-heart infusion;

— yeast extract.

A.4 Agar

Bacteriological agar.

A.5 Other

— Egg yolk emulsion;

— skimmed milk powder;

— whole, defibrinated or lysed blood, blood powder, plasma, fibrinogen, haemin, from specified animals;

— ox bile for bacteriology;

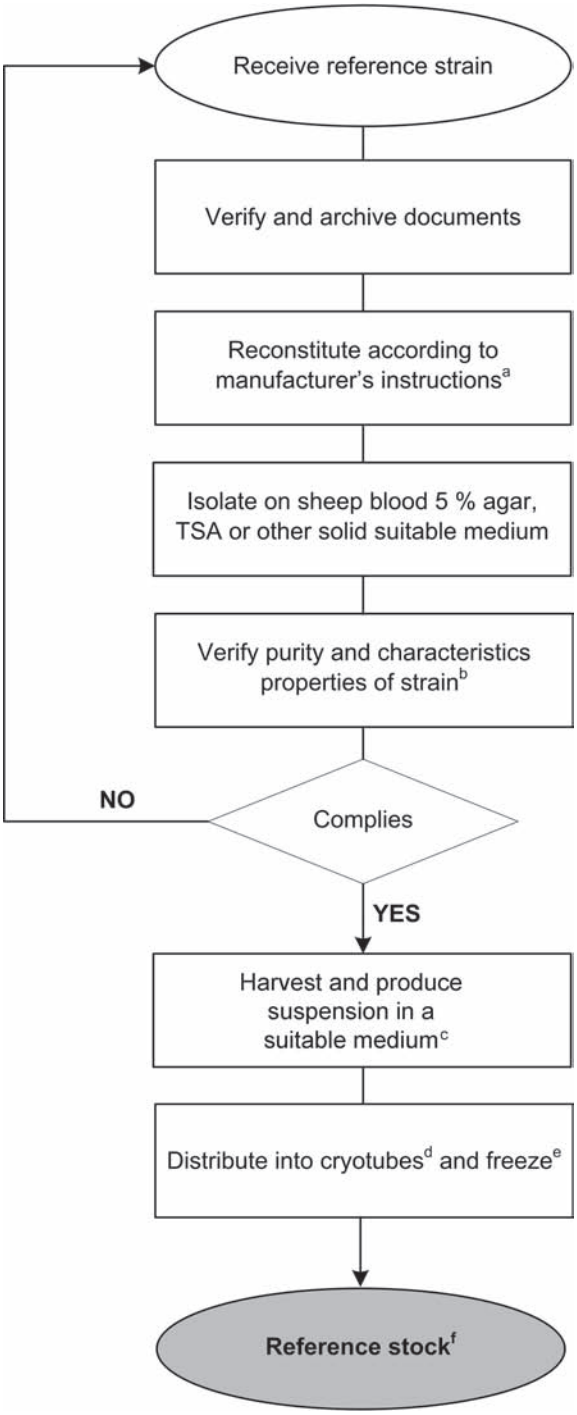
— bile salts.

NOTE This includes bile salts no. 3.

Annex B
(normative)

Preparation of reference stock and working culture

B.1 Preparation of reference stock from a reference strain



a In general, resuspension in a nutrient broth and holding time for resuscitation.

b Verify morphology of colonies and Gram staining or identify using biochemical tests.

c For example a cryoprotective medium, such as TSB supplemented with 10 % to 15 % glycerol volume fraction.

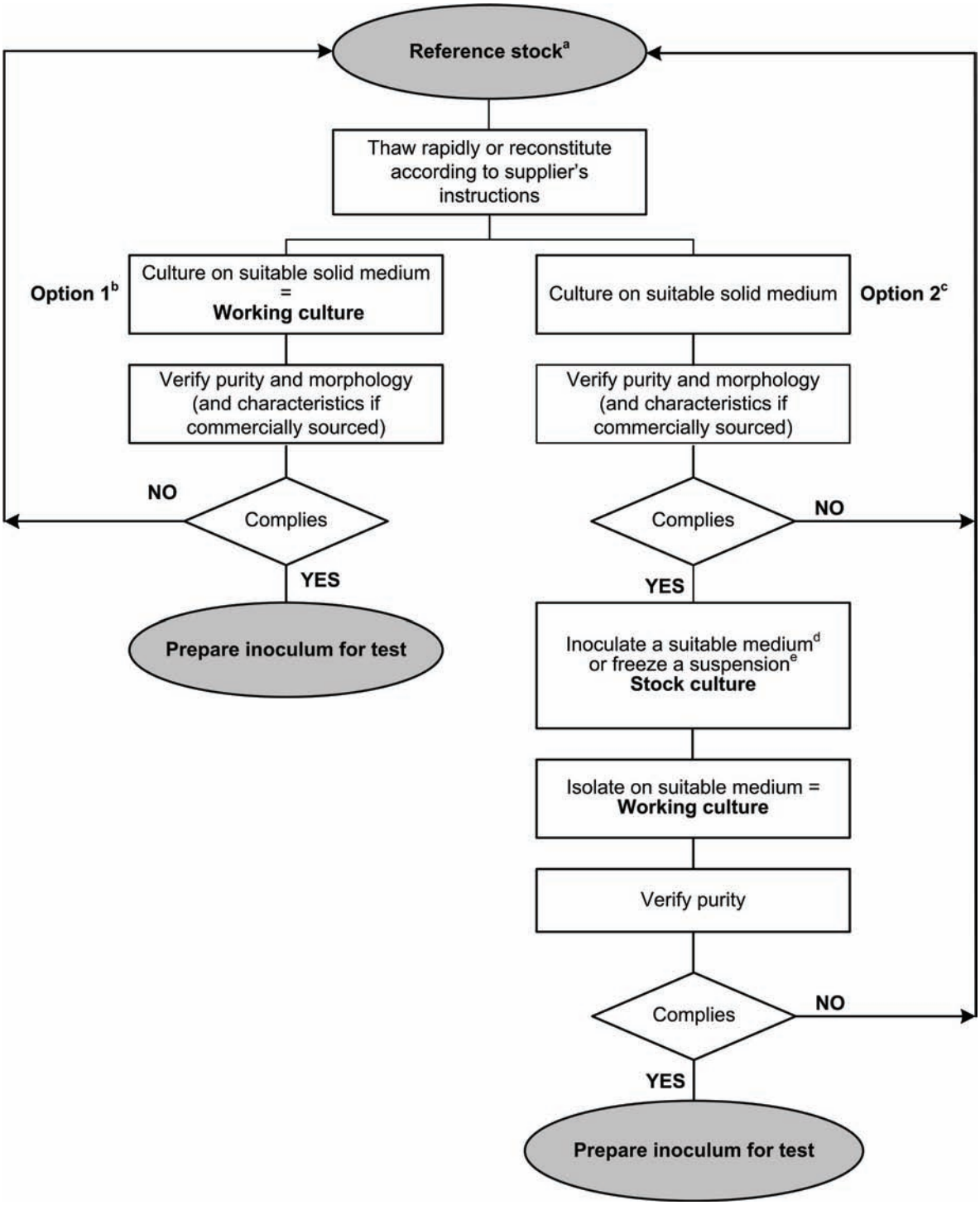
d Cryotubes may contain beads.

e Freezing at a temperature below −70 °C enables extended storage. Storage life at a higher temperature is limited.[36]

f May also be used directly as a working culture.

Figure B.1 — Flow chart of preparation of reference stock from reference strain

B.2 Preparation of working culture from reference stock



a Verify and archive documents, including check on traceability to reference strain and relevant characteristics, if reference stock is obtained from outside source.

b This procedure is preferable.

c This procedure may be necessary for some strains, e.g. for quantitative tests. Document all stages.

d For example inoculate a slant of TSA or sheep blood TSA or other suitable medium, incubate for 24 h 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.[36]

e For example a cryoprotective medium, such as TSB supplemented with 10 % to 15 % volume fraction glycerol. Freezing at a temperature below −70 °C enables extended storage. Storage life at a higher temperature is limited.[36]

Figure B.2 — Flow chart of preparation of working culture from reference stock

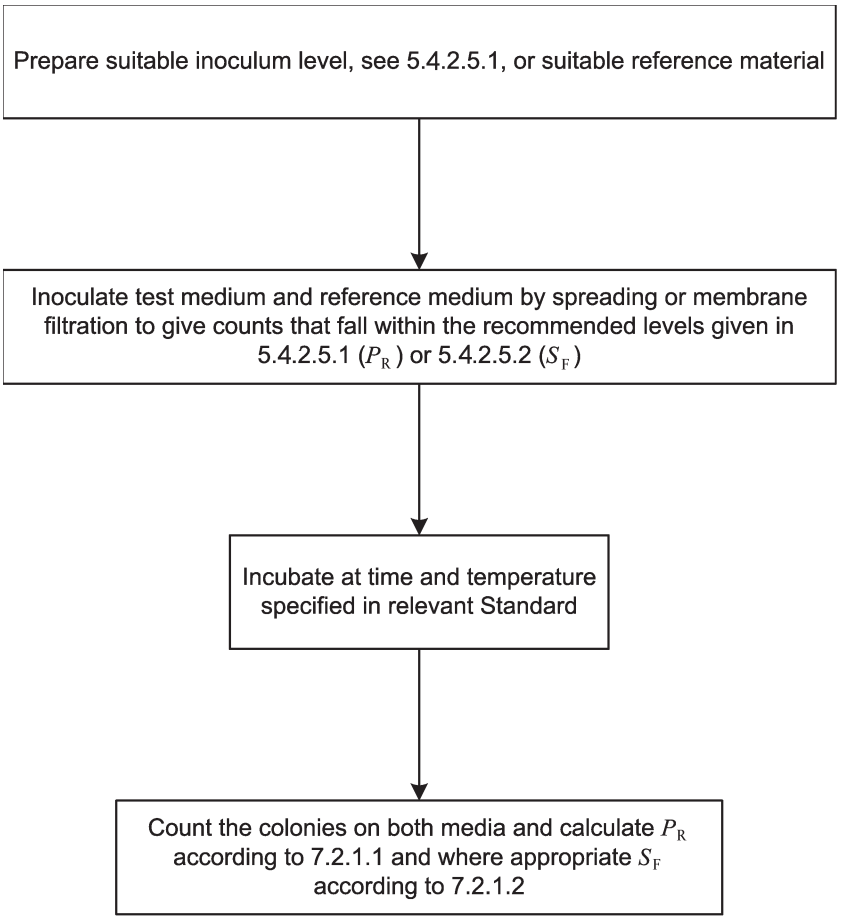
Annex C
(normative)

Flow charts of methods for performance testing

C.1 General

See Clause 7.

C.2 Quantitative method for solid culture media: productivity and selectivity (see 7.2.2 and Figure C.1)



Key

P_R productivity ratio

S_F selectivity factor

The productivity ratio, P_R, may be used to compare:

a) a non-selective medium with a non-selective reference medium;

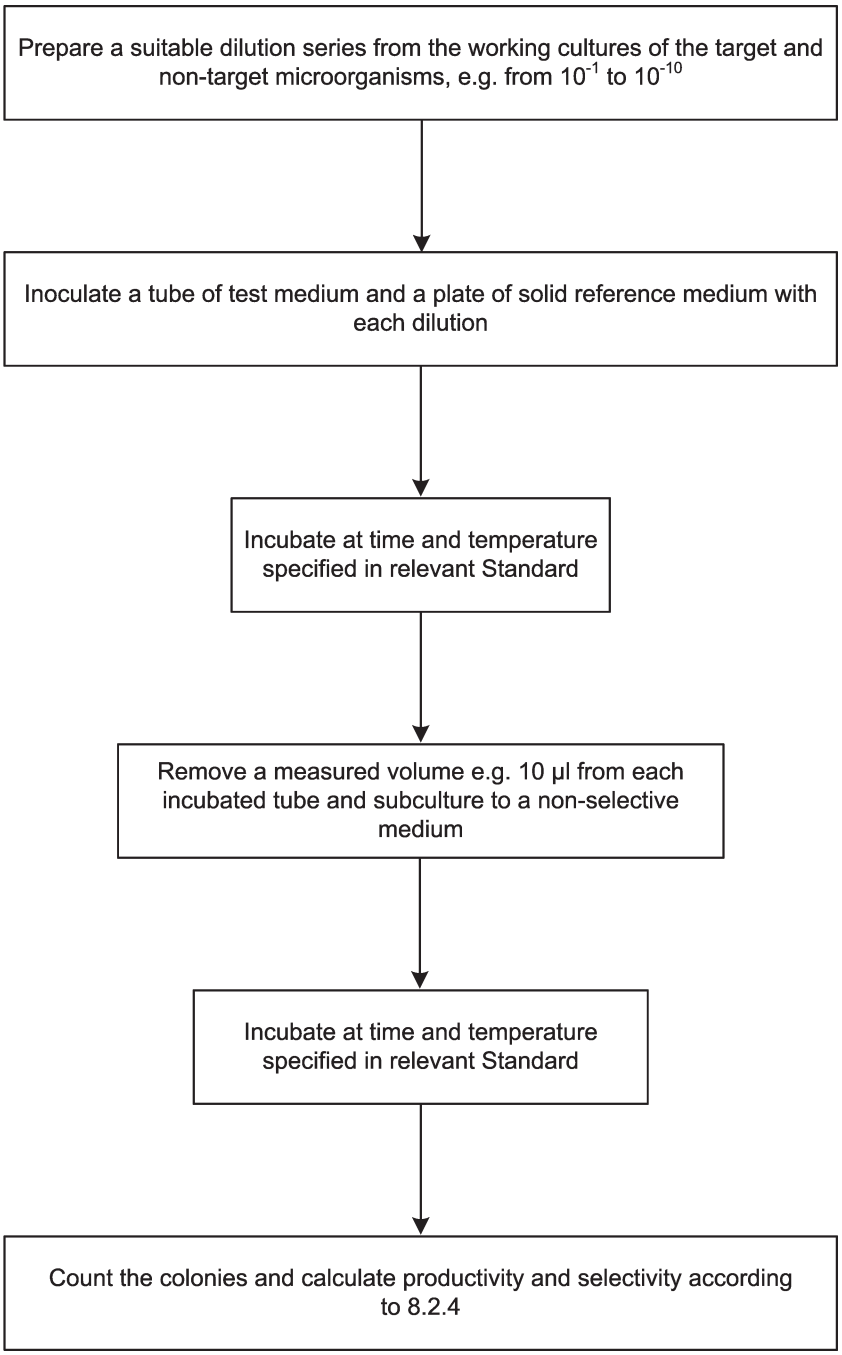
b) a selective medium with a non-selective reference medium;

c) a selective medium with a selective reference medium.

For incubation (third level/box of this figure), see 5.4.2.6.

Figure C.1 — Flow chart for quantitative testing of solid culture media

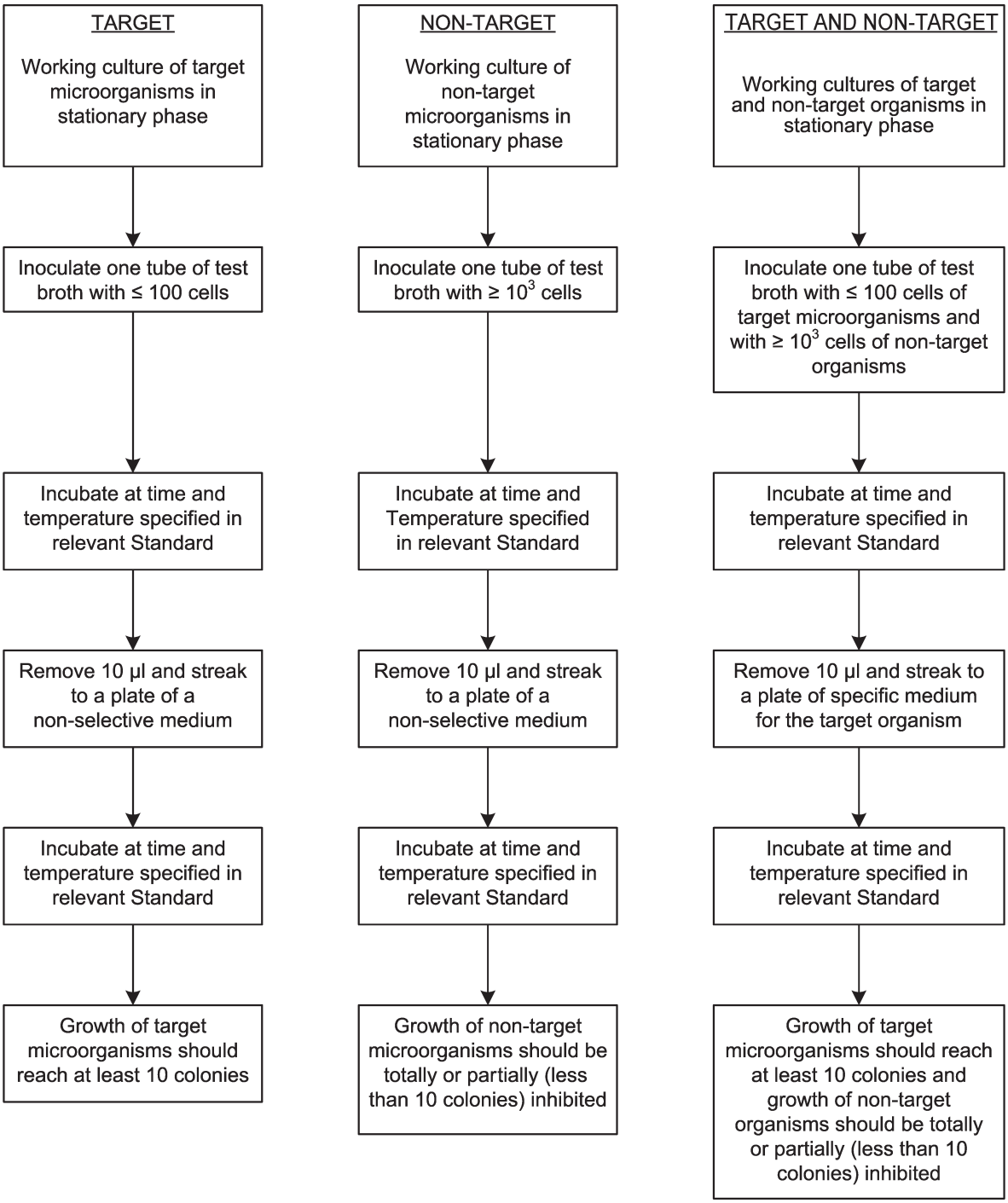
C.3 Quantitative tube method for performance testing of liquid enrichment media — Dilution to extinction method (see 8.2 and Figure C.2)



Note For incubation (fifth level/box of this figure), see 5.4.2.6.

Figure C.2 — Flow chart for performance testing of liquid enrichment media (dilution to extinction method)

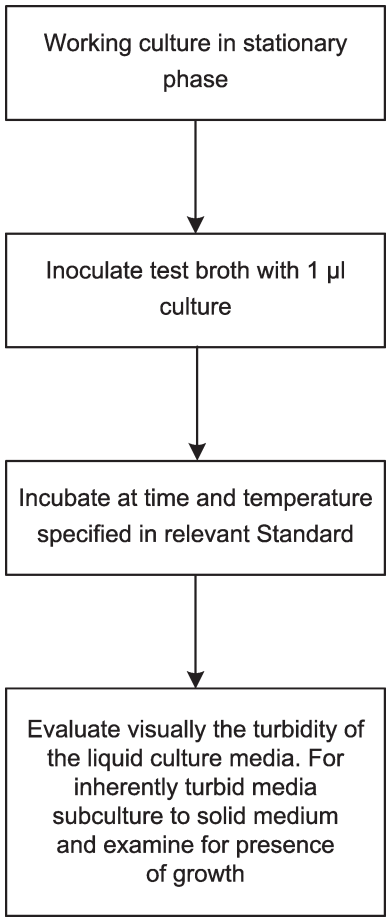
C.4 Qualitative single tube method for selective liquid enrichment media (with target, non-target, or a mixture of target and non-target microorganisms in the same tube) (see 8.3 and Figure C.3)



Note For incubation (fifth level/box of each column of this figure), see 5.4.2.6.

Figure C.3 — Flow chart for qualitative single tube method for selective liquid enrichment media

C.5 Qualitative single tube method for non-selective and selective liquid media: turbidity (see 8.4 and Figure C.4)



Check, in general, the characteristics of bacterial growth for each liquid medium, e.g. gas production, colour change, if applicable.

Note For incubation (third level/box of this figure), see 5.4.2.6.

Figure C.4 — Flow chart for qualitative testing of liquid media using a single tube (turbidity)

Annex D
(informative)

Example of card for recording test results of culture media

Table D.1 — Example of a card

Control card for internal quality testing of culture media				
culture medium:		volume prepared:	pouring date:	internal batch number:
dehydrated medium (and code):	Supplier:	Batch	amount:	date/signature:
Supplement:	Supplier:	Batch	amount:	date/signature:
Process details:				
Physical quality control				
Expected appearance of dehydrated culture medium	Colour normal free-flowing	quality confirmed: yes ☐ no ☐	defects:	date/signature:
expected pH-value:	measured pH:	quality confirmed: yes ☐ no ☐	defects:	date/signature:
expected quantity filled and/or layer thickness:	observed:	quality confirmed: yes ☐ no ☐	defects:	date/signature:
expected colour:	observed:	quality confirmed: yes ☐ no ☐	defects:	date/signature:
expected clarity/presence of optical artefacts:	observed:	quality confirmed: yes ☐ no ☐	defects:	date/signature:
expected gel stability/ consistency/moisture:	observed:	quality confirmed: yes ☐ no ☐	defects:	date/signature:
Microbial contamination				
No. of tested plates or tubes: Incubation:	result:	quality confirmed: yes ☐ no ☐	No. of contaminated plates or tubes:	date/signature:
Microbiological growth — Productivity		Method of control:	Quantitative ☐	Qualitative ☐
strains: incubation: reference medium:	criteria:	result:	quality confirmed: yes ☐ no ☐	date/signature:
Microbiological growth — Selectivity		Method of control:	Quantitative ☐	Qualitative ☐
strains: incubation: reference medium:	criteria:	result:	quality confirmed: yes ☐ no ☐	date/signature:

Table D.1 *(continued)*

Microbiological growth — Specificity		Method of control: Quantitative ☐ Qualitative ☐		
strains: incubation: reference medium:	criteria:	result:	quality confirmed: yes ☐ no ☐	date/signature:
Release of the batch				
Details of storage:		release of the batch yes ☐ no ☐		date/signature:

Annex E
(normative)

Test microorganisms and performance criteria for culture media commonly used in food microbiology

This annex gives information on the culture medium, culture conditions, test microorganisms, culture collection number of test organisms and the expected reactions when performance testing of culture media is carried out.

Specific strains have been selected for testing in order to ensure consistency between laboratories and to facilitate the demonstration of differences between media (batch to batch, between manufacturers). These strains have been fully evaluated to ensure their suitability and consistency in performance.

Where more than one strain is listed for each aspect of performance testing (productivity, selectivity, specificity), the minimum strains to be used have been indicated by the letter b. Commercial or non-commercial suppliers are expected to use additional strains e.g. those shown in Table E.1 to further ensure the quality of the culture media they supply.

Table E.1 has been established taking into account the control strains used in the European Pharmacopoeia (EP) and the recommendations for culture media for food microbiology from the Working Party of the International Committee on Food Microbiology and Hygiene (ICFMH). These criteria shall be included in specific International Standards when prepared or revised in the future. A validated batch of culture medium is one which has shown satisfactory performance. The strain numbers specified in Table E.1 are those from the catalogue of universal strain identifiers compiled by the World Data Centre for Microorganisms (WDCM).[20] This catalogue contains details of the reference strains represented by each WDCM number and contact details of the culture collections. All cited media are described within European and International Standards.

If strain variability is encountered, investigate the effect of the culture medium (e.g. by obtaining the same medium from a different manufacturer), and obtain an additional reference culture from the culture collection in which it was originally deposited. Users are requested to give relevant feedback on strain variability to WG 5, *Culture media*, of ISO/TC 34/SC 9 through the secretariat of ISO/TC 34/SC 9.

The footnotes used in Table E.1 are the following:

a Full names of media abbreviated terms are given in Table E.2.

b Strains to be used as a minimum.

c Make reference to the reference strain catalogue available on http://www.wfcc.info for information on culture collection strain numbers and contact details.

d Strain free of choice; one of the strains has to be used as a minimum.

e L: liquid medium, S: solid medium, SS: semi-solid medium.

f Growth/turbidity is categorized as: 0 — no growth/no turbidity; 1 — weak growth/slight turbidity; 2 — growth/good turbidity (see 7.4.1.2, 8.4.1).

g *Escherichia coli* WDCM 00013 is given by the specific standard.

h *Escherichia coli* WDCM 00013 is a strong β-d-glucuronidase producer and WDCM 00202 is a weak β-d-glucuronidase producer.

i Some national restrictions and directions may require the use of a different serovar. Make reference to national requirements relating to the choice of *Salmonella* serovars.

j In case of both quantitative and qualitative use for the medium, only results of the quantitative tests are required (see Table E.1).

k More details for quality control of MSRV medium including final concentration of the inoculum and criteria are given in ISO 6579.

l If nutrient agar is used for two or three of these different applications: perform the *Salmonella* growth test as a minimum (if laboratory tests for this organism).

m If BPW is used for two or three of these different applications: perform the *Salmonella* enrichment test as a minimum (if laboratory tests for this organism).

n Choose the strain(s) according to the method for which TSA is used as a reference medium.

Table E.1 — Test microorganisms and performance criteria for culture media commonly used in food microbiology

Selective media for enumeration of microorganisms											
Media[a]	**Type**[e]	**Microorganism**	**International Standard**	**Function**	**Incubation**	**Control strain**	**WDCM number**[c]	**Reference media**	**Method of control**	**Criteria**	**Characteristic reaction**
Agar Listeria according to Ottaviani and Agosti	S	*Listeria monocytogenes*	ISO 11290-2	Productivity	(44 ± 4) h/ (37 ± 1) °C	*Listeria monocytogenes* 4b *Listeria monocytogenes* 1/2a	00021[b] 00109	TSA	Quantitative	$P_R \geq 0,5$	Blue green colonies with opaque halo
				Selectivity		*Escherichia coli*[d] *Enterococcus faecalis*[d]	00012 or 00013 00009 or 00087	—	Qualitative	Total inhibition (0)	—
				Specificity		*Listeria innocua*	00017	—	Qualitative	—	Blue green colonies without opaque halo
Baird-Parker	S	Coagulase-positive staphylococci	ISO 6888-1	Productivity	(24 ± 2) h to (48 ± 2) h/ (37 ± °1) °C	*Staphylococcus aureus*	00034[b] 00032	TSA	Quantitative	$P_R \geq 0,5$	Black or grey colonies with clear halo (egg yolk clearing reaction)
				Selectivity	(48 ± 2) h/ (37 ± 1) °C	*Escherichia coli*[d]	00012 00013	—	Qualitative	Total inhibition (0)	—
				Specificity	(24 ± 2) h to (48 ± 2) h/ (37 ± 1) °C	*Staphylococcus saprophyticus* *Staphylococcus epidermidis*	00159[b] 00036	—	Qualitative	—	Black or grey colonies without egg yolk clearing reaction
BGBLB	L	Coliforms	ISO 4831	Productivity	(24 ± 2) h to (48 ± 2) h/ (30 ± 1) °C	*Escherichia coli* *Citrobacter freundii*	00012[b] 00013 00006	—	Qualitative	Turbidity (2)[f] and gas in Durham tube	Gas production and turbidity
				Selectivity		*Enterococcus faecalis*[d]	00009 00087	—	Qualitative	Partial inhibition without gas production	—
CFC	S	*Pseudomonas spp.*	ISO 13720	Productivity	(44 ± 4) h/ (25 ± 1) °C	*Pseudomonas fluorescens* *Pseudomonas fragi*	00115[b] 00116	TSA	Quantitative	$P_R \geq 0,5$	—
				Selectivity		*Escherichia coli*[d]	00012 00013	—	Qualitative	Total inhibition (0)	—

Table E.1 *(continued)*

DG18	S	Yeasts and moulds	ISO 21527-2	Productivity	5 d/ (25 ± 1) °C	*Saccharomyces cerevisiae* *Wallemia sebi* *Aspergillus restrictus* *Eurotium rubrum*	00058[b] 00182[b] 00183 00184	SDA	Quantitative	$P_R \geq 0,5$	Characteristic colony/propagules according to each species
				Selectivity		*Escherichia coli* *Bacillus subtilis subsp. spizizenii*	00012 or 00013[g] 00003	—	Qualitative	No growth	—
DRBC	S	Yeasts and moulds	ISO 21527-1	Productivity	5 days/ (25 ± 1) °C	*Saccharomyces cerevisiae* *Aspergillus brasiliensis* *Candida albicans* *Mucor racemosus*	00058[b] 00053[b] 00054 00181	SDA	Quantitative	$P_R \geq 0,5$	Characteristic colony/propagules according to each species
				Selectivity		*Escherichia coli* *Bacillus subtilis subsp. spizizenii*	00012 or 00013[g] 00003	—	Qualitative	No growth	—
EC	L	*Escherichia coli*	ISO 7251	Productivity	(24 ± 2) h to (48 ± 2) h/ (44 ± 1) °C	*Escherichia coli*	00012[b] 00013	—	Qualitative	Turbidity (2)[f] and gas in Durham tube	Gas production and turbidity
				Selectivity		*Pseudomonas£ aeruginosa*	00025	—	Qualitative	No growth	—
IS ("TS")	S	Sulfite-reducing bacteria	ISO 15213	Productivity	(24 ± 3) h to (48 ± 2) h/ (37 ± 1) °C anaerobic atmosphere	*Clostridium perfringens*	00007[b] 00080	TSA or other non-selective medium for anaerobes	Quantitative	$P_R \geq 0,5$	Black colonies
				Specificity		*Escherichia coli*[d]	00012 00013	—	Qualitative	—	No blackening

Table E.1 *(continued)*

LST	L	Coliforms	ISO 4831	Productivity	(24 ± 2) h to (48 ± 2) h/ (30 ± 1) °C	*Escherichia coli* *Citrobacter freundii*	00012[b] 00013 00006	—	Qualitative	Turbidity (2)[f] and gas in Durham tube	Gas production and turbidity
				Selectivity		*Enterococcus faecalis*[d]	00009 00087	—	Qualitative	No growth	—
		Escherichia coli	ISO 7251	Productivity	(24 ± 2) h to (48 ± 2) h/ (37 ± 1) °C	*Escherichia coli*	00012[b] 00013	—	Qualitative	Turbidity (2)[fi] and gas in Durham tube	Gas production and turbidity
				Selectivity		*Enterococcus faecalis*[d]	00009 00087	—	Qualitative	No growth	—
mCCDA	S	*Campylobacter*	ISO 10272-2	Productivity	(44 ± 4) h/ (41,5 ± 1) °C microaerobic atmosphere	*Campylobacter jejuni*[d] *Campylobacter coli*[d]	00156 00005 00004	Blood agar	Quantitative	$P_R \geq 0,5$	Greyish, flat and moist, sometimes with metallic sheen
				Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	—	—
						Staphylococcus aureus	00034	—	Qualitative	—	—
MRS	S	Lactic acid bacteria	ISO 15214	Productivity	(72 ± 3) h/ (30 ± 1) °C	*Lactobacillus sakei* *Lactococcus lactis* *Pediococcus pentosaceus*	00015[b] 00016[b] 00158	Media batch MRS already validated	Quantitative	$P_R \geq 0,7$	Characteristic colonies according to each species
				Selectivity	(72 ± 3) h/ (30 ± 1) °C	*Escherichia coli*[d] *Bacillus cereus*	00012 or 00013 00001	—	Qualitative	Total inhibition (0)	—
MYP	S	*Bacillus cereus*	ISO 7932	Productivity	(24 ± 3) h to (44 ± 4) h/ (30 ± 1) °C	*Bacillus cereus*	00001	TSA	Quantitative	$P_R \geq 0,5$	Pink colonies with precipitation halo
				Selectivity	(44 ± 4) h/ (30 ± 1) °C	*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Total inhibition (0)	—
				Specificity		*Bacillus subtilis subsp. spizizenii*	00003	—	Qualitative	—	Yellow colonies without precipitation halo

Table E.1 *(continued)*

RPFA	S	Coagulase-positive staphylococci	ISO 6888-2	Productivity	(24 ± 2) h to (48 ± 2) h/ (37 ± 1) °C	*Staphylococcus aureus*	00034[b] 00032	TSA	Quantitative	$P_R \geq 0,5$	Black or grey colonies with opacity halo
				Selectivity	(48 ± 2) h/ (37 ± 1) °C	*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Total inhibition (0)	—
				Specificity	(24 ± 2) h to (48 ± 2) h/ (37 ± 1) °C	*Staphylococcus saprophyticus* *Staphylococcus epidermidis*	00159[b] 00036	—	Qualitative	—	Black or grey colonies without opacity halo
PPA	S	*Pseudomonas spp.*	ISO/ TS 11059	Productivity	(48 ± 2) h/ (25 ± 1) °C	*Pseudomonas fluorescens* *Pseudomonas aeruginosa*	00115[b] 00025	TSA	Quantitative	$P_R \geq 0,5$	—
				Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Total inhibition (0)	—
TBX	S	β-D-Glucuronidase-positive *Escherichia coli*	ISO 16649-1 and ISO 16649-2	Productivity	(21 ± 3) h/ (44 ± 1) °C	*Escherichia coli*[h]	00012[d] 00013[d] 00202[b]	TSA	Quantitative	$P_R \geq 0,5$	Blue colonies
				Selectivity		*Enterococcus faecalis*[d]	00009 00087		Qualitative	Total inhibition (0)	—
				Specificity		*Citrobacter freundii* *Pseudomonas aeruginosa*	00006[b] 00025		Qualitative	—	White to green-beige colonies
TSC (SC)	S	*Clostridium perfringens*	ISO 7937	Productivity	(20 ± 2) h/ (37 ± 1) °C anaerobic atmosphere	*Clostridium perfringens*	00007[b] 00080	TSA or other non-selective medium for anaerobes	Quantitative	$P_R \geq 0,5$	Black colonies
				Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Total inhibition (0)	—
						Pseudomonas aeruginosa	00025				
VRBG	S	*Enterobacteriaceae*	ISO 21528-2	Productivity	(24 ± 2) h/ (37 ± 1) °C	*Escherichia coli* *Salmonella* Typhimurium[d,i] *Salmonella* Enteritidis[d,i]	00012[b] 00013 00031 00030	TSA	Quantitative	$P_R \geq 0,5$	Pink to red colonies with or without precipitation halo
				Selectivity		*Enterococcus faecalis*[d]	00009 00087	—	Qualitative	Total inhibition (0)	—

Table E.1 *(continued)*

VRBL	S	Coliforms	ISO 4832	Productivity	(24 ± 2) h/ (30 ± 1) °C	*Escherichia coli*	00012[b] 00013	TSA	Quantitative	$P_R \geq 0,5$	Purplish-red colonies with or without precipitation halo
				Selectivity		*Enterococcus faecalis*[d]	00009 00087	—	Qualitative	Total inhibition (0)	—
				Specificity		*Pseudomonas aeruginosa*	00025		Qualitative	—	Colourless to beige colonies
Non-selective media for enumeration of microorganisms											
Media[a]	**Type**[e]	**Micro-organisms**	**International Standard**	**Function**	**Incubation**	**Control strains**	**WDCM numbers**[c]	**Reference media**	**Method of control**	**Criteria**	**Characteristic reactions**
PCA MPCA	S	Colony count	ISO 4833	Productivity	(72 ± 3) h/ (30 ± 1) °C	*Bacillus subtilis subsp. spizizenii*	00003[b]	TSA	Quantitative	$P_R \geq 0,7$	—
						Escherichia coli	00012[b] 00013				
						Staphylococcus aureus	00034				
Selective enrichment media											
Media[a]	**Type**[e]	**Micro-organisms**	**International Standard**	**Function**	**Incubation**	**Control strains**	**WDCM numbers**[c]	**Reference media**	**Method of control**	**Criteria**	**Characteristic reactions of target microorganism**
Bolton	L	*Campylobacter*	ISO 10272-1	Productivity	(5 ± 1) h/ (37 ± 1) °C then (44 ± 4) h/ (41,5 ± 1) °C microaerobic atmosphere	*Campylobacter jejuni*[d] *Campylobacter coli*	00156 or 00005 00004	—	Qualitative	> 10 colonies on mCCDA	Greyish, flat and moist, sometimes with metallic sheen
						+ *Escherichia coli*[d]	00012 00013				
						+ *Proteus mirabilis*	00023				
				Selectivity		*Escherichia coli*[d]	00012 00013	—	Qualitative	Total inhibition (0) on TSA	—
						Proteus mirabilis	00023				

Table E.1 *(continued)*

EE	L	*Enterobacteriaceae*	ISO 21528-1	Productivity	(24 ± 2) h/ (37 ± 1) °C	*Escherichia coli*	00012[b] 00013	—	Qualitative	> 10 colonies on VRBG	Pink to red colonies with or without precipitation halo
						+ *Enterococcus faecalis*[d]	00009 or 00087				
						Salmonella Typhimurium[i] *Salmonella* Enteritidis[i]	00031 or 00030				
						+ *Enterococcus faecalis*[d]	00009 or 00087				
				Selectivity		*Enterococcus faecalis*[d]	00009 or 00087	—	Qualitative	Total inhibition (0) on TSA	—
Fraser	L	*Listeria monocytogenes*	ISO 11290-1	Productivity	(48 ± 2) h/ (37 ± 1) °C	*Listeria monocytogenes* 4b	00021[b]	—	Qualitative	> 10 colonies on Agar Listeria according to Ottaviani and Agosti	Blue green colonies with opaque halo
						+ *Escherichia coli*[d]	00012 or 00013				
						+ *Enterococcus faecalis*[d]	00009 or 00087				
						Listeria monocytogenes 1/2a	00109				
						+ *Escherichia coli*[d]	00012 or 00013				
						+ *Enterococcus faecalis*[d]	00009 or 00087				
				Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Total inhibition (0) on TSA	—
						Enterococcus faecalis[d]	00009 or 00087	—	Qualitative	< 100 colonies on TSA	—
Giolitti Cantoni	L	Coagulase-positive staphylococci	ISO 6888-3	Productivity	(24 ± 2) h to (48 ± 2) h/ (37 ± 1) °C	*Staphylococcus aureus*	00034[b]	—	Qualitative	> 10 colonies on Baird Parker or RPFA	Characteristic colonies according to each medium (see ISO 6888-1 for Baird Parker and ISO 6888-2 for RPFA)
						+ *Escherichia coli*[d]	00012 or 00013				
						Staphylococcus aureus	00032				
						+ *Escherichia coli*[d]	00012 or 00013				
				Selectivity	(48 ± 2) h/ (37 ± 1) °C	*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Total inhibition (0) on TSA	—

Table E.1 *(continued)*

Half-Fraser	L	*Listeria monocytogenes*	ISO 11290-1	Productivity	(24 ± 2) h/ (30 ± 1) °C	*Listeria monocytogenes* 4b	00021[b]	—	Qualitative	> 10 colonies on Agar Listeria according to Ottaviani and Agosti	Blue green colonies with opaque halo
						+ *Escherichia coli*[d]	00012 or 00013				
						+ *Enterococcus faecalis*[d]	00009 or 00087				
						Listeria monocytogenes 1/2a	00109				
						+ *Escherichia coli*[d]	00012 or 00013				
						+ *Enterococcus faecalis*[d]	00009 or 00087				
				Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Total inhibition (0) on TSA	—
						Enterococcus faecalis[d]	00009 or 00087	—	Qualitative	< 100 colonies on TSA	—
ITC	L	*Yersinia enterocolitica*	ISO 10273	Productivity	(44 ± 4) h/ (25 ± 1) °C	*Yersinia enterocolitica*	00038[b]	—	Qualitative	> 10 colonies on CIN or SSDC	Characteristic colonies according to each medium (see ISO 10273)
						+ *Escherichia coli*[d]	00012 or 00013				
						+ *Pseudomonas aeruginosa*	00025				
				Selectivity		*Pseudomonas aeruginosa*	00025	—	Qualitative	Total inhibition (0) on TSA	—
						Proteus mirabilis	00023				
MKTTn	L	*Salmonella*	ISO 6579	Productivity	(24 ± 3) h/ (37 ± 1) °C	*Salmonella* Enteritidis[d,i] *Salmonella* Typhimurium[d,i]	00030 00031	—	Qualitative	> 10 colonies on XLD or other medium of choice	Characteristic colonies according to each medium (see ISO 6579)
						+ *Escherichia coli*[d]	00012 or 00013				
						+ *Pseudomonas aeruginosa*	00025				
				Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Partial inhibition ≤ 100 colonies on TSA	—
						Enterococcus faecalis[d]	00009 or 00087	—	Qualitative	< 10 colonies on TSA	—

Table E.1 *(continued)*

MSRV[k]	SS	*Salmonella*	ISO 6579	Productivity	2 × (24 ± 3) h/ (41,5 ± 1) °C	*Salmonella* Enteritidis[d,i]	00030	—	Qualitative	Grey-white, turbid zone extending out from inoculated drop(s). After 24–48 h, the turbid zone(s) will be (almost) fully migrated over the plate.	Possible extra: characteristic colonies after subculturing on XLD[k]
						Salmonella Typhimurium[d,i]	00031				
				Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Possible growth at the place of the inoculated drop(s) without a turbid zone.	—
						Enterococcus faecalis[d]	00009 or 00087	—	Qualitative	No growth	—
MMG	L	β-D-Glucuronidase-positive *E. coli*	ISO 16649-3	Productivity	(24 ± 2) h/ (37 ± 1) °C	*Escherichia coli*	00012[b] 00013	—	Qualitative	Acid production	Colour change to yellow
				Selectivity		*Enterococcus faecalis*[d]	00009 or 00087	—	Qualitative	No growth	—
PSB	L	*Yersinia enterocolitica*	ISO 10273	Productivity	3 to 5 days/ (25 ± 1) °C	*Yersinia enterocolitica*	00038[b]	—	Qualitative	> 10 colonies on CIN or SSDC	Characteristic colonies according to each medium (see ISO 10273)
						+ *Escherichia coli*[d]	00012 or 00013				
						+ *Pseudomonas aeruginosa*	00025				
						Yersinia enterocolitica	00160				
						+ *Escherichia coli*[d]	00012 or 00013				
						+ *Pseudomonas aeruginosa*	00025				
				Selectivity		*Pseudomonas aeruginosa*	00025	—	Qualitative	Total inhibition (0) on TSA	—
						Proteus mirabilis	00023				

Table E.1 *(continued)*

RVS	L	*Salmonella*	ISO 6579	Productivity	(24 ± 3) h/ (41,5 ± 1) °C	*Salmonella* Enteritidis[d,i] *Salmonella* Typhimurium[d,i] + *Escherichia coli* + *Pseudomonas aeruginosa*	00030 00031 00012 or 00013 00025	—	Qualitative	> 10 colonies on XLD or other medium of choice	Characteristic colonies according to each medium (see ISO 6579)
				Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Partial inhibition ≤ 100 colonies on TSA	—
						Enterococcus faecalis[d]	00009 or 00087	—	Qualitative	< 10 colonies on TSA	—
TSPB	L	*Bacillus cereus*	ISO 21871	Productivity	(48 ± 4) h/ (30 ± 1) °C	*Bacillus cereus*	00001	—	Qualitative	> 10 colonies on PEMBA or MYP	Characteristic colonies according to each medium (see ISO 21871)
				Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Total inhibition (0) on TSA	—
Non-selective liquid media											
Media[a]	**Type**[e]	**Micro-organisms**	**International Standard**	**Function**	**Incubation**	**Control strains**	**WDCM numbers**[c]	**Reference media**	**Method of control**	**Criteria**	**Characteristic reactions**
BHI	L	Coagulase-positive staphylococci	ISO 6888-1 ISO 6888-3	Productivity	(24 ± 2) h/ (37 ± 1) °C	*Staphylococcus aureus*	00034	—	Qualitative	Turbidity (1–2)[f]	—
Brucella	L	*Campylobacter*	ISO 10272 (all parts)	Productivity	2 to 5 days/ (41,5 ± 1) °C microaerobic atmosphere	*Campylobacter jejuni*[d] *Campylobacter coli*[d]	00156 00005 00004	—	Qualitative	Turbidity (1–2)[f]	—
Diluents for special purposes e.g. BPW with bromo-cresol-purple	L	Dilution liquids	ISO 6887 (all parts)	Diluent	45 min – 1 h/ 20 °C to 25 °C	*Escherichia coli*[d] *Staphylococcus aureus*	00012 or 00013 00034[b]	TSA	Quantitative	±30 % colonies/ T_0 (±30 % of original count)	—

Table E.1 *(continued)*

Quarter-strength Ringer's Peptone solution Peptone-salt Phosphate buffer solution	L	Dilution liquids	ISO 6887 (all parts)	Diluent	45 min – 1 h/ 20 °C to 25 °C	*Escherichia coli*[d] *Staphylococcus aureus*	00012 or 00013 00034[b]	TSA	Quantitative	±30 % colonies/ T_0 (±30 % of original count)	—
Thioglycollate	L	*Clostridium perfringens*	ISO 7937	Productivity	(21 ± 3) h/ (37 ± 1) °C	*Clostridium perfringens*	00007	—	Qualitative	Turbidity (1–2)[f]	—
TSYEB	L	*Listeria monocytogenes*	ISO 11290 (all parts)	Productivity	(21 ± 3) h/ (25 ± 1) °C	*Listeria monocytogenes* 4b *Listeria monocytogenes* 1/2a	00021[b] 00109	—	Qualitative	Turbidity (1–2)[f]	—
Selective isolation media											
Media[a]	**Type**[e]	**Micro-organisms**	**International Standard**	**Function**	**Incubation**	**Control strains**	**WDCM numbers**[c]	**Reference media**	**Method of control**	**Criteria**	**Characteristic reactions**
Agar Listeria according to Ottaviani and Agosti[j]	S	*Listeria monocytogenes*	ISO 11290-1	Productivity	(44 ± 4) h/ (37 ± 1) °C	*Listeria monocytogenes* 4b *Listeria monocytogenes* 1/2a	00021[b] 00109	—	Qualitative	Good growth (2)	Blue green colonies with opaque halo
				Selectivity		*Escherichia coli*[d] *Enterococcus faecalis*[d]	00012 or 00013 00009 or 00087	—	Qualitative	Total inhibition (0)	—
				Specificity		*Listeria innocua*	00017	—	Qualitative	—	Blue green colonies without opaque halo
mCCDA[j]	S	*Campylobacter*	ISO 10272 (all parts)	Productivity	(44 ± 4) h/ (41,5 ± 1) °C microaerobic atmosphere	*Campylobacter jejuni*[d] *Campylobacter coli*[d]	00156 00005 00004	—	Qualitative	Good growth (2)	Greyish, flat and moist, sometimes with metallic sheen
				Selectivity		*Escherichia coli*[d]	000012 or 00013	—	Qualitative	Total or partial inhibition (0 – 1)	No characteristic colonies
						Staphylococcus aureus	00034	—	Qualitative	Total inhibition (0)	—

Table E.1 *(continued)*

CT-SMAC	S	*Escherichia coli* O157	ISO 16654	Productivity	(21 ± 3) h/ (37 ± 1) °C	*Escherichia coli* O157:H7	00014 (non-toxigenic strain)	—	Qualitative	Good growth (2)	Transparent colonies with a pale yellowish-brown appearance and a diameter ~1 mm
				Selectivity		*Staphylococcus aureus*[d]	00032 or 00034	—	Qualitative	Total inhibition (0)	—
						Escherichia coli[d]	00012 or 00013	—	Qualitative	Partial inhibition (1)	Growth of some pink colonies
CIN SSDC	S	*Yersinia enterocolitica*	ISO 10273	Productivity	(21 ± 3) h/ (30 ± 1) °C	*Yersinia enterocolitica*	00038[b] 00160	—	Qualitative	Good growth (2)	Characteristic colonies according to each medium (see ISO 10273)
				Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Total or partial inhibition (0 – 1)	No characteristic colonies
						Staphylococcus aureus	00034	—	Qualitative	Total inhibition (0)	—
CPC mCPC	S	*Vibrio spp.* other than *Vibrio parahaemolyticus/V. cholerae*	ISO/ TS 21872-2	Productivity	(24 ± 3) h/ (37 ± 1) °C	*Vibrio vulnificus*	00187[b]	—	Qualitative	Good growth (2)	Yellow colonies surrounded by a yellow coloration in the medium
						Vibrio cholerae non-O1/non-O139	00203[b]	—	Qualitative	Good growth (2)	Purple colonies surrounded by a purple coloration in the medium
				Selectivity		*Escherichia coli*[d]	00012 or 00013 or 00090	—	Qualitative	Total inhibition (0)	—
MYP[j]	S	*Bacillus cereus*	ISO 21871	Productivity	(21 ± 3) h to 48 h/ (30 ± 1) °C	*Bacillus cereus*	00001	—	Qualitative	Good growth (2)	Pink colonies with precipitation halo
				Selectivity	(44 ± 4) h/ (30 ± 1) °C	*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Total inhibition (0)	—
				Specificity		*Bacillus subtilis subsp. spizizenii*	00003	—	Qualitative	—	Yellow colonies without precipitation halo
PEMBA	S	*Bacillus cereus*	ISO 21871	Productivity	(21 ± 3) h to (44 ± 4) h/ (37 ± 1) °C	*Bacillus cereus*	00001	—	Qualitative	Good growth (2)[i]	Turquoise-blue colonies with precipitation halo
				Selectivity	(44 ± 4) h/ (37 ± 1) °C	*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Total inhibition (0)	—
				Specificity		*Bacillus subtilis subsp. spizizenii*	00003	—	Qualitative	—	White colonies without precipitation halo

Table E.1 *(continued)*

Media[a]	Type[e]	Micro-organisms	International Standard	Function	Incubation	Control strains	WDCM numbers[c]	Reference media	Method of control	Criteria	Characteristic reactions
SDS	S	*Vibrio spp.* other than *Vibrio parahaemolyticus/V. cholerae*	ISO/ TS 21872-2	Productivity	(24 ± 3) h/ (37 ± 1) °C	*Vibrio vulnificus*	00187[b]	—	Qualitative	Good growth (2)	Purple/green colonies with an opaque halo
						Vibrio cholerae non-O1/non-O139	00203[b]	—	Qualitative	Good growth (2)	Yellow colonies with an opaque halo
				Selectivity		*Escherichia coli*[d]	00012 or 00013 or 00090	—	Qualitative	Total inhibition (0)	—
TBX[j]	S	β-D-Glucuronidase-positive *Escherichia coli*	ISO 16649-3	Productivity Selectivity	(21 ± 3) h/ (44 ± 1) °C	*Escherichia coli*[h]	00012[d] 00013[d] 00202[b]	—	Qualitative	Good growth (2)	Blue colonies
						Enterococcus faecalis[d]	00009 or 00087	—	Qualitative	Total inhibition (0)	—
				Specificity		*Citrobacter freundii*	00006[b]	—	Qualitative	—	White to green-beige colonies
						Pseudomonas aeruginosa	00025				
VRBG[j]	S	*Enterobacteriaceae*	ISO 21528-1	Productivity	(24 ± 2) h/ (37 ± 1) °C	*Escherichia coli*	00012[b] 00013	—	Qualitative	Good growth (2)	Pink to red colonies with or without precipitation halo
						Salmonella Typhimurium[i]	00031 or 00032				
						Salmonella Typhimurium[i]					
				Selectivity		*Enterococcus faecalis*[d]	00009 or 00087	—	Qualitative	Total inhibition (0)	—
XLD	S	*Salmonella*	ISO 6579	Productivity	(24 ± 3) h/ (37 ± 1) °C	*Salmonella* Typhimurium[d,i]	00031	—	Qualitative	Good growth (2)	Colonies with black centre and a lightly transparent zone of reddish colour due to the colour change of the medium
						Salmonella Enteritidis[d,i]	00030				
				Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Growth or partial inhibition (0 – 1)	Yellow colonies
						Enterococcus faecalis[d]	00009 or 00087	—	Qualitative	Total inhibition (0)	—
Non-selective isolation media											

Table E.1 *(continued)*

Nutrient agar[l]	S	*Entero-bacteriaceae*	ISO 21528 (all parts)	Productiv-ity	(24 ± 2) h/ (37 ± 1) °C	*Escherichia coli*	00012[b] 00013	—	Qualitative	Good growth (2)	—
		Salmonella	ISO 6579		(24 ± 2) h/ (37 ± 1) °C	*Salmonella* Typhimurium[d,i] *Salmonella* Enteritidis[d,i]	00030 00031				
		Yersinia entero-colitica	ISO 10273		(24 ± 2) h/ (30 ± 1) °C	*Yersinia enterocolitica*	00038[b] 00160				
TSYEA	S	*Listeria mono-cytogenes*	ISO 11290 (all parts)	Productiv-ity	(21 ± 3) h/ (37 ± 1) °C	*Listeria monocytogenes* 4[b] *Listeria monocytogenes* 1/2a	00021b 00109	—	Qualitative	Good growth (2)	—
Multipurpose media											
Media[a]	**Type[e]**	**Micro-organisms**	**Interna-tional Standard**	**Function**	**Incubation**	**Control strains**	**WDCM num-bers[c]**	**Reference media**	**Method of control**	**Criteria**	**Characteristic reactions**
BPW[m]	L	Diluent for all enumerations of microorgan-isms	ISO 6887 (all parts), ISO 6887-5	Dilution	45 min – 1 h/ 20 °C to 25 °C	*Escherichia coli* *Staphylococcus aureus*	00012[b] 00013 00034[b]	TSA	Quantita-tive	±30 % colonies/ T_0 (±30 % of original count)	—
		Diluent for *Listeria monocytogenes* enumeration	ISO 11290-2	Dilution	(1 h ± 5°min) / (20 ± 2) °C	*Listeria monocytogenes* 4b *Listeria monocytogenes* 1/2a	00021[b] 00109	TSA	Quantita-tive	±30 % colonies/ T_0 (±30 % of original count)	—
		Pre-enrichment for *Salmonella* detection	ISO 6579	Productiv-ity	(18 ± 2) h/ (37 ± 1) °C	*Salmonella* Typhimurium[d,i] *Salmonella* Enteritidis[d,i]	00031 00030	—	Qualitative	Turbidity (1–2)[f]	—
		Pre-enrichment for *Entero-bacteriaceae* detection	ISO 21528-1	Productiv-ity	(18 ± 2) h/ (37 ± 1) °C	*Escherichia coli* *Salmonella* Typhimurium[i] *Salmonella* Enteritidis[i]	00012[b] 00013 00031 or 00030	—	Qualitative	Turbidity (1–2)[f]	—
Reference media for enumeration of microorganisms											
Media[a]	**Type[e]**	**Micro-organisms**	**Interna-tional Standard**	**Function**	**Incubation**	**Control strains**	**WDCM num-bers[c]**	**Reference media**	**Method of control**	**Criteria**	**Characteristic reactions**
Blood agar	S	*Campylobacter*	ISO 10272-2	Productiv-ity	(44 ± 4) h/ (41,5 ± 1) °C	*Campylobacter jejuni*[d] *Campylobacter coli*[d]	00156 00005 00004	Media batch blood agar already validated	Quantita-tive	$P_R \geq 0,7$	—

Table E.1 *(continued)*

TSA[n]	S	Colony count	—	Productivity	As specified in the method in which TSA is used as reference medium	*Bacillus cereus* *Bacillus subtilis subsp. spizizenii* *Escherichia coli* *Escherichia coli* O157:H7 *Listeria monocytogenes* 4b *Staphylococcus aureus*	00001 00003 00012 00014 (non-toxigenic strain) 00021 00034	Media batch TSA already validated	Quantitative	$P_R \geq 0,7$	Characteristic colony according to each species
SDA	S	Colony count	—	Productivity	As specified in the method in which SDA is used as reference medium	*Saccharomyces cerevisiae* *Aspergillus brasiliensis*	00058[b]	Media batch SDA already validated	Quantitative	$P_R \geq 0,7$	Characteristic colony/propagules/germs according to each species

[a] Full names of media abbreviated terms are given in Table E.2.

[b] Strains to be used as a minimum.

[c] Make reference to the reference strain catalogue available on http://www.wfcc.info for information on culture collection strain numbers and contact details.

[d] Strain free of choice; one of the strains has to be used as a minimum.

[e] L: liquid medium, S: solid medium, SS: semi-solid medium.

[f] Growth/turbidity is categorized as: 0 — no growth/no turbidity; 1 — weak growth/slight turbidity; 2 — growth/good turbidity (see 7.4.1.2, 8.4.1).

[g] *Escherichia coli* WDCM 00013 is given by the specific standard.

[h] *Escherichia coli* WDCM 00013 is a strong β-d-glucuronidase producer and WDCM 00202 is a weak β-d-glucuronidase producer.

[i] Some national restrictions and directions may require the use of a different serovar. Make reference to national requirements relating to the choice of *Salmonella* serovars.

[j] In case of both quantitative and qualitative use for the medium, only results of the quantitative tests are required (see Table E.1).

[k] More details for quality control of MSRV medium including final concentration of the inoculum and criteria are given in ISO 6579.

[l] If nutrient agar is used for two or three of these different applications: perform the *Salmonella* growth test as a minimum (if laboratory tests for this organism).

[m] If BPW is used for two or three of these different applications: perform the *Salmonella* enrichment test as a minimum (if laboratory tests for this organism).

[n] Choose the strain(s) according to the method for which TSA is used as a reference medium.

Table E.2 — Abbreviated terms for media used in Table E.1

Abbreviated media term	Full name of the media	International Standard
Baird –Parker	Baird-Parker agar	ISO 6888-1
BGBLB	Brilliant green lactose bile broth	ISO 4831
BHI	Brain heart infusion broth	ISO 6888-1 and ISO 6888-3
Bolton	Bolton broth	ISO 10272-1
BPW	Buffered peptone water	ISO 6887 (all parts) ISO 6579 ISO 11290-2 ISO 21528-1
Brucella	Brucella broth	ISO 10272 (all parts)
CFC	Cephalothin fucidin cetrimide agar	ISO 13720
CIN	Cefsulodin, Irgasan novobiocin agar	ISO 10273
CPC	Cellobiose polymyxin B colistin agar	ISO/TS 21872-2
CT-SMAC	Cefixime tellurite sorbitol MacConkey agar	ISO 16654
DG18	Dichloran glycerol agar	ISO 21527-2
DRBC	Dichloran-rose bengal chloramphenicol agar	ISO 21527-1
EC	EC broth	ISO 7251
EE	Buffered brilliant green bile glucose broth	ISO 21528-1
Fraser	Fraser broth	ISO 11290-1
Half-Fraser	Half Fraser broth	ISO 11290-1
IS (“TS”)	Iron sulfite agar (“Tryptose sulfite agar”)	ISO 15213
ITC	Irgasan, ticarcillin chlorate broth	ISO 10273
LST	Lauryl sulfate broth, lauryl tryptose broth	ISO 4831 and ISO 7251
mCCDA	Modified charcoal cefoperazone deoxycholate agar	ISO 10272 (all parts)
mCPC	Modified cellobiose polymyxin B colistin agar	ISO/TS 21872-2
MKTTn	Muller-Kauffmann tetrathionate novobiocin broth	ISO 6579
MMG	Minerals-modified glutamate medium	ISO 16649-3
MPCA	Plate count agar with skimmed milk/ milk plate count agar	ISO 4833
MRS	MRS medium (de Man, Rogosa and Sharpe)	ISO 15214
MSRV	Modified semi-solid Rappaport-Vassiliadis medium	ISO 6579
MYP	Mannitol egg yolk polymyxin agar	ISO 7932
PCA	Plate count agar	ISO 4833
PEMBA	Polymyxin pyruvate egg yolk mannitol bromothymol blue agar	ISO 21871
PPA	Penicillin and pimaricin agar	ISO/TS 11059
PSB	Peptone, sorbitol and bile salts broth	ISO 10273
RPFA	Rabbit plasma fibrinogen agar	ISO 6888-2

Table E.2 *(continued)*

Abbreviated media term	Full name of the media	International Standard
RVS	Rappaport-Vassiliadis soya peptone broth	ISO 6579
SDA	Sabouraud dextrose agar	—
SDS	Sodium dodecyl sulfate polymyxin sucrose agar	ISO/TS 21872-2
SSDC	Salmonella Shigella deoxycholate calcium agar	ISO 10273
TBX	Tryptone bile X-glucuronide agar	ISO 16649 (all parts)
TCBS	Thiosulfate citrate bile salts sucrose agar	ISO/TS 21872-1
Thioglycollate	Fluid thioglycollate medium	ISO 7937
TSA	Tryptone soya agar	—
TSC/SC	Sulfite cycloserine agar/ tryptose sulphite cycloserine agar without egg yolk	ISO 7937
TSPB	Tryptone soya polymyxin broth	ISO 21871
TSYEA	Tryptone soya yeast extract agar	ISO 11290 (all parts)
TSYEB	Tryptone soya yeast extract broth	ISO 11290 (all parts)
VRBG	Violet red bile glucose agar	ISO 21528 (all parts)
VRBL	Violet red bile lactose agar	ISO 4832
XLD	Xylose lysine deoxycholate agar	ISO 6579

Annex F
(normative)

Test microorganisms and performance criteria for culture media commonly used in water microbiology

Specific strains have been selected for testing in order to ensure consistency between laboratories and to facilitate the demonstration of differences between media (batch to batch, between manufacturers). The strains specified in Table F.1 have been fully evaluated to ensure their suitability and consistency in performance.

Where more than one strain is listed for each aspect of performance testing (productivity, selectivity, specificity), the minimum strains to be used have been indicated by the letter b. Commercial or non-commercial suppliers are expected to use additional strains e.g. those shown in Table F.1 to further ensure the quality of the culture media they supply.

These criteria shall be included in specific standards when prepared or revised in the future. A validated batch of media is one which has shown satisfactory performance. The strain numbers specified in Table F.1 are those from the catalogue of universal strain identifiers compiled by the World Data Centre for Microorganisms (WDCM).[20] This catalogue contains details of the reference strains represented by each WDCM number and contact details of the culture collections. All cited media are described within EN and ISO standards.

If strain variability is encountered, investigate the effect of the culture medium (e.g. by obtaining the same medium from a different manufacturer), and obtain an additional reference culture from the culture collection in which it was originally deposited. Users are requested to feed back relevant information on strain variability to WG 5, *Culture media*, of ISO/TC 34/SC 9 through the secretariat of ISO/TC 34/SC 9.

The footnotes used in Table F.1 are the following:

a Full names of media abbreviated terms are given in Table F.2.

b Strains to be used as a minimum.

c Make reference to the reference strain catalogue available on http://www.wfcc.info for information on culture collection strain numbers and contact details.

d Strain free of choice; one of the strains has to be used as a minimum.

e L: liquid medium, S: solid medium, SS: semi-solid medium.

f More details for quality control of *Legionella* media including storage of the control strains are given in ISO 11731.

g More details for quality control and quality criteria of MUG/EC medium are given in ISO 9308-3:1998, Annex E; selectivity is not specified in the standard.

h More details for quality control and quality criteria of MUD/SF medium are given in ISO 7899-1:1998, Annex E.

i Some national restrictions and directions may require the use of a different serovar. Make reference to national requirements relating to the choice of *Salmonella* serovars.

j Growth/turbidity is categorized as: 0 — no growth/turbidity; 1 — weak growth/turbidity; 2 — good growth/turbidity (see 7.4.1.2, 8.4.1).

k If BPW is used for two of these different applications: perform the *Salmonella* enrichment test as a minimum (if laboratory tests for this organism).

l Choose the strain(s) according to the method for which TSA is used as a reference medium.

Table F.1 — Test microorganisms and performance criteria for culture media commonly used in water microbiology

Selective media for enumeration of microorganisms by comparing with a non-selective reference medium											
Media[a]	**Type**[e]	**Micro-organisms**	**International Standard**	**Function**	**Incubation**	**Control strains**	**WDCM numbers**[c]	**Reference media**	**Method of control**	**Criteria**	**Characteristic reactions**
Colilert	L	*Escherichia coli* /coliform bacteria	ISO 9308-2	Productivity	(20 ± 2)°h/ (36 ± 2) °C	*Escherichia coli*	00013[b] 00090	TSA	Quantitative	$P_R \geq 0,5$	Yellow colour and fluorescence for *E. coli*
						Klebsiella pneumoniae	00206	TSA	Quantitative	$P_R \geq 0,5$	Yellow colour equal or greater than the comparator for coliform bacteria
				Selectivity		*Pseudomonas aeruginosa*[d]	00207 or 00025	—	Qualitative	Total inhibition (0)	Less yellow than the comparator
GVPC[f]	S	*Legionella*	ISO 11731 and ISO 11731-2	Productivity	2-5 days/ (36 ± 2) °C	*Legionella pneumophila*	00107[b] 00180	BCYE	Quantitative	$P_R \geq 0,5$	White-grey-blue-purple colonies with an entire edge and exhibiting a characteristic ground-glass appearance
					5-10 days/ (36 ± 2) °C	*Legionella anisa*	00106				
				Selectivity	3 days/ (36 ± 2) °C	*Enterococcus faecalis*[d]	00009 or 00087	—	Qualitative	Total inhibition (0)	—
						Pseudomonas aeruginosa[d]	00026 or 00025	—	Qualitative	Total or partial inhibition (0-1)	—
						Escherichia coli[d]	00012 or 00013				
Lactose TTC	S	*Escherichia coli* / coliform bacteria	ISO 9308-1	Productivity	(21 ± 3) h/ (36 ± 2) °C	*Escherichia coli*	00179[b] 00012 00013	TSA	Quantitative	$P_R \geq 0,5$	Yellow colour in the medium under the membrane
						Enterobacter aerogenes	00175				
						Citrobacter freundii	00006				
				Selectivity		*Enterococcus faecalis*[d]	00009 or 00087	—	Qualitative	Total inhibition (0)	—
				Specificity		*Pseudomonas aeruginosa*[d]	00025 or 00026	—	Qualitative	—	Red colonies, blue colour in the medium
mCP	S	*Clostridium perfringens*	Council Directive 98/83/EC	Productivity	(21 ± 3) h/ (44 ± 1) °C anaerobic atmosphere	*Clostridium perfringens*	00007[b] 00080 00174	TSA or other non-selective medium for anaerobes	Quantitative	$P_R \geq 0,5$	Yellow colonies; Phophatase test positive
				Specificity		*Clostridium bifermentans*	00079	—	Qualitative	—	Blue colonies; Phosphatase test negative
				Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Total inhibition (0)	—

Table F.1 *(continued)*

Media[a]	Type[e]	Micro-organisms	International Standard	Function	Incubation	Control strains	WDCM numbers[c]	Reference media	Method of control	Criteria	Characteristic reactions
Selective media for enumeration of microorganisms by comparing with a previously accepted batch (for use in special cases)											
Pseudomonas CN	S	*Pseudomonas aeruginosa*	ISO 16266	Productivity	(44 ± 4) h/(36 ± 2) °C	*Pseudomonas aeruginosa*	00024[b] 00025 00026	TSA	Quantitative	$P_R \geq 0,5$	Blue-green colonies with fluorescence under UV light (360 ± 20 nm)
				Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Total inhibition (0)	—
						Enterococcus faecalis[d]	00009 or 00087				
Slanetz and Bartley	S	Intestinal enterococci	ISO 7899-2	Productivity	(44 ± 4) h/(36 ± 2) °C	*Enterococcus faecalis*	00009[b] 00087 00176	TSA	Quantitative	$P_R \geq 0,5$	Red-maroon-pink colonies
						Enterococcus faecium[d]	00177 00178				
				Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Total inhibition (0)	—
						Staphylococcus aureus[d]	00032 or 00034				
Sulfite Iron Tryptose Sulfite (TS)	S	Sulfite-reducing anaerobes (clostridia)	ISO 6461-2	Productivity	(44 ± 4) h/(37 ± 1) °C anaerobic atmosphere	*Clostridium perfringens*	00007[b] 00080	TSA or Blood agar or other non-selective medium for anaerobes	Quantitative	$P_R \geq 0,5$	Black colonies
				Specificity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	—	No blackening
TSC	S	*Clostridium perfringens*	ISO 14189	Productivity	(21 ± 3) h/(44 ± 1) °C anaerobic atmosphere	*Clostridium perfringens*	00007[b] 00080 00174	TSA or Blood agar or other non-selective medium for anaerobes	Quantitative	$P_R \geq 0,5$	Black colonies
				Selectivity		*Bacillus subtilis subsp. spizizenii*	00003	—	Qualitative	Total inhibition (0)	—

Table F.1 *(continued)*

Colilert	L	*Escherichia coli* /coliform bacteria	ISO 9308-2	Productivity	(20 ± 2)h/ (36±2) °C	*Escherichia coli*	00013[b] 00090	previously validated batch Colilert	Quantitative	$P_R \geq 0,7$	Yellow colour and fluorescence for *E. coli*
						Klebsiella pneumoniae	00206	previously validated batch Colilert	Quantitative	$P_R \geq 0,7$	Yellow colour equal or greater than the comparator for coliform bacteria
				Selectivity		*Pseudomonas aeruginosa*[d]	00207 or 00025	—	Qualitative	Total inhibition (0)	Less yellow than the comparator
GVPC[f]	S	*Legionella*	ISO 11731 and ISO 11731-2	Productivity	2-5 days/ (36 ± 2) °C	*Legionella pneumophila*	00107[b] 00180	Media batch GVPC already validated	Quantitative	$P_R \geq 0,7$	White-grey-blue-purple colonies with an entire edge and exhibiting a characteristic ground-glass appearance
					5-10 days/ (36 ± 2) °C	*Legionella anisa*	00106				
				Selectivity	3 days/ (36 ± 2) °C	*Enterococcus faecalis*[d]	00009 or 00087	—	Qualitative	Total inhibition (0)	—
						Pseudomonas aeruginosa[d]	00026 or 00025	—	Qualitative	Total or partial inhibition (0 - 1)	—
						Escherichia coli[d]	00012 or 00013				
Lactose TTC	S	*Escherichia coli* / coliform bacteria	ISO 9308-1	Productivity	(21 ± 3) h/ (36 ± 2) °C	*Escherichia coli*	00179[b] 00012 00013	Media batch Lactose TTC already validated	Quantitative	$P_R \geq 0,7$	Yellow colour in the medium under the membrane
						Enterobacter aerogenes	00175				
						Citrobacter freundii	00006				
				Selectivity		*Enterococcus faecalis*[d]	00009 or 00087	—	Qualitative	Total inhibition (0)	—
				Specificity		*Pseudomonas aeruginosa*[d]	00025 or 00026	—	Qualitative	—	Red colonies, blue colour in the medium
mCP	S	*Clostridium perfringens*	Council Directive 98/83/EC	Productivity	(21 ± 3) h/ (44 ± 1) °C anaerobic atmosphere	*Clostridium perfringens*	00007[b] 00080 00174	Media batch mCP already validated	Quantitative	$P_R \geq 0,7$	Yellow colonies; Phophatase test positive
				Specificity		*Clostridium bifermentans*	00079	—	Qualitative	—	Blue colonies; Phosphatase test negative
				Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Total inhibition (0)	—

Table F.1 *(continued)*

Pseudomonas CN	S	*Pseudomonas aeruginosa*	ISO 16266	Productivity	(44 ± 4) h/ (36 ± 2) °C	*Pseudomonas aeruginosa*	00024[b] 00025 00026	Media batch Pseudomonas CN already validated	Quantitative	$P_R \geq 0,7$	Blue-green colonies with fluorescence under UV light (360 ± 20 nm)
				Selectivity		*Escherichia coli*[d] *Enterococcus faecalis*[d]	00012 or 00013 00009 or 00087	—	Qualitative	Total inhibition (0)	—
Slanetz and Bartley	S	Intestinal enterococci	ISO 7899-2	Productivity	(44 ± 4) h/ (36 ± 2) °C	*Enterococcus faecalis* *Enterococcus faecium*[d]	00009[b] 00087 00176 00177 00178	Media batch Slanetz and Bartley already validated	Quantitative	$P_R \geq 0,7$	Red-maroon-pink colonies
				Selectivity		*Escherichia coli*[d] *Staphylococcus aureus*[d]	00012 or 00013 00032 or 00034	—	Qualitative	Total inhibition (0)	—
Sulfite Iron Tryptose Sulfite (TS)	S	Sulfite-reducing anaerobes (clostridia)	ISO 6461-2	Productivity	(44 ± 4) h/ (37 ± 1) °C anaerobic atmosphere	*Clostridium perfringens*	00007[b] 00080	Media batch Sulfite iron or TS already validated	Quantitative	$P_R \geq 0,7$	Black colonies
				Specificity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	—	No blackening
TSC	S	*Clostridium perfringens*	ISO 14189	Productivity	(21 ± 3) h/ (44 ± 1) °C anaerobic atmosphere	*Clostridium perfringens*	00007[b] 00080 00174	Media batch TSC already validated	Quantitative	$P_R \geq 0,7$	Black colonies
				Selectivity		*Bacillus subtilis subsp. spizizenii*	00003	—	Qualitative	Total inhibition (0)	—
Non-selective media for enumeration of microorganisms											
Media[a]	**Type**[e]	**Micro-organisms**	**International Standard**	**Function**	**Incubation**	**Control strains**	**WDCM numbers**[c]	**Reference media**	**Method of control**	**Criteria**	**Characteristic reactions**
YEA	S	Total flora	ISO 6222	Productivity	(44 ± 4) h/ (36 ± 2) °C	*Escherichia coli*[d] *Bacillus subtilis subsp. spizizenii*	00012 or 00013 00003	Media batch YEA already validated	Quantitative	$P_R \geq 0,7$	—
Selective enrichment media											

Table F.1 *(continued)*

Media[a]	Type[e]	Micro-organisms	International Standard	Function	Incubation	Control strains	WDCM numbers[c]	Reference media	Method of control	Criteria	Characteristic reactions
Bolton Preston	L	*Campylobacter*	ISO 17995	Productivity	(44 ± 4) h/ (37 ± 1) °C microaerobic atmosphere	*Campylobacter jejuni*[d] *Campylobacter coli*[d]	00156 00005 00004	—	Qualitative	> 10 colonies on mCCDA	Small, flat or convex colonies with a glossy surface
						+ *Escherichia coli*[d]	00012 or 00013				
						+ *Proteus mirabilis*	00023				
				Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Total inhibition on TSA (0)	—
						Proteus mirabilis	00023				
MUG/EC[g]	L	*Escherichia coli* / coliform bacteria	ISO 9308-3	Productivity	48 h/ (44 ± 0,5) °C	*Escherichia coli*	00179	Details for method of control and quality criteria of MUG/EC medium are given in ISO 9308-3:1998, Annex E.			
MUD/SF[h]	L	Intestinal enterococci	ISO 7899-1	Productivity	(44 ± 4) h/ (44 ± 0,5) °C	*Enterococcus faecalis*	00176	Details for method of control and quality criteria of MUD/SF medium are given in ISO 7899-1:1998, Annex E.			
						Enterococcus hirae	00089				
						Enterococcus faecium	00178				
				Selectivity		*Aerococcus viridans*	00061				
						Lactococcus lactis	00016				
						Staphylococcus epidermidis	00132				
RVS	L	*Salmonella*	ISO 19250	Productivity	(24 ± 3) h/ (41,5 ± 1) °C	*Salmonella* Enteritidis[d,i] *Salmonella* Typhimurium[d,i]	00030 00031	—	Qualitative	> 10 colonies on XLD or other medium of choice	Characteristic colonies according to each medium (see standard)
						+ *Escherichia coli*[d]	00012 or 00013				
						+ *Pseudomonas aeruginosa*	00025				
				Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Partial inhibition ≤ 100 colonies on TSA	—
						Enterococcus faecalis[d]	00009 or 00087	—	Qualitative	< 10 colonies on TSA	—
Non-selective liquid media											

Table F.1 *(continued)*

Media[a]	Type[e]	Micro-organisms	International Standard	Function	Incubation	Control strains	WDCM numbers[c]	Reference media	Method of control	Criteria	Characteristic reactions
DRCM	L	Sulfite-reducing anaerobes (clostridia)	ISO 6461-1	Productivity	(44 ± 4) h/ (36 ± 1) °C anaerobic atmosphere	*Clostridium perfringens*	00007[b] 00080	—	Qualitative	Turbidity (1-2)[j]	Blackening
				Specificity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Turbidity (0-1)[j]	No blackening
Saline salt Peptone diluent Peptone salt solution Ringer's solution (1/4 strength) Phosphate buffer solution	L	Dilution liquids	ISO 8199	Diluent	45 min – 1 h/ 20 °C – 25 °C	*Escherichia coli*[d] *Staphylococcus aureus*	00012 or 00013 00034	TSA	Quantitative	+/- 30 % colonies/ T_0 (+/- 30 % of original count)	—
Selective isolation media											
Media[a]	**Type[e]**	**Micro-organisms**	**International Standard**	**Function**	**Incubation**	**Control strains**	**WDCM numbers[c]**	**Reference media**	**Method of control**	**Criteria**	**Characteristic reactions**
mCCDA	S	*Campylobacter*	ISO 17995	Productivity	(44 ± 4) h/ (41,5 ± 1) °C microaerobic atmosphere	*Campylobacter jejuni*[d] *Campylobacter coli*[d]	00156 or 00005 00004	—	Qualitative	Good growth (2)	Small, flat or convex colonies with a glossy surface
				Selectivity		*Escherichia coli*[d]	00012 or 00013 or 00179 or 00090	—	Qualitative	Total or partial inhibition (0–1)	No characteristic colonies
						Staphylococcus aureus[d]	00032 or 00034	—	Qualitative	Total inhibition (0)	—

Table F.1 *(continued)*

XLD	S	*Salmonella*	ISO 19250	Productivity	(24 ± 3) h/ (36 ± 2) °C	*Salmonella* Typhimurium[d,i]	00031	—	Qualitative	Good growth (2)	Colonies with black centre and a lightly transparent zone of reddish colour due to the colour change of the medium
						Salmonella Enteritidis[d,i]	00030				
				Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Growth or partial inhibition (0–1)	Yellow colonies
						Enterococcus faecalis[d]	00009 or 00087	—	Qualitative	Total inhibition (0)	—
Multipurpose media											
Media[a]	**Type[e]**	**Micro-organisms**	**International Standard**	**Function**	**Incubation**	**Control strains**	**WDCM numbers[c]**	**Reference media**	**Method of control**	**Criteria**	**Characteristic reactions**
BPW[k]	L	Diluent for enumerations of all microorganisms	ISO 6887	Dilution	45 min – 1 h/ 20 °C to 25 °C	*Escherichia coli*[d]	00012 or 00013	TSA	Quantitative	+/- 30 % colonies/T_0 (+/- 30 % of original count)	—
						Staphylococcus aureus	00034				
		Pre-enrichment for *Salmonella* detection	ISO 19250	Productivity	(18 ± 2) h/ (36 ± 2) °C	*Salmonella* Typhimurium[d,i]	00031	—	Qualitative	Turbidity (1-2)[j]	—
						Salmonella Enteritidis[d,i]	00030				
Reference media for enumeration of microorganisms											
Media[a]	**Type[e]**	**Micro-organisms**	**International Standard**	**Function**	**Incubation**	**Control strains**	**WDCM numbers[c]**	**Reference media**	**Method of control**	**Criteria**	**Characteristic reactions**
BCYE	S	Colony count	ISO 11731 and ISO 11731-2	Productivity	2-5 days / (36 ± 2) °C	*Legionella pneumophila*	00107[b]	Media batch BCYE already validated	Quantitative	$P_R \geq 0,7$	White-grey-blue-purple colonies with an entire edge and exhibiting a characteristic ground-glass appearance

Table F.1 *(continued)*

TSA[l]	S	Colony count	—	Productivity	As specified in the method in which TSA is used as reference medium	*Escherichia coli*[d]	00012 00013 00090 00179	Media batch TSA already validated	Quantitative	$P_R \geq 0,7$	Characteristic colony according to each species
						Clostridium perfringens	00007				
						Pseudomonas aeruginosa	00024				
						Enterococcus faecalis	00087				

[a] Full names of media abbreviated terms are given in Table F.2.

[b] Strains to be used as a minimum.

[c] Make reference to the reference strain catalogue available on http://www.wfcc.info for information on culture collection strain numbers and contact details.

[d] Strain free of choice; one of the strains has to be used as a minimum.

[e] L: liquid medium, S: solid medium, SS: semi-solid medium.

[f] More details for quality control of *Legionella* media including storage of the control strains are given in ISO 11731.

[g] More details for quality control and quality criteria of MUG/EC medium are given in ISO 9308-3:1998, Annex E; selectivity is not specified in the standard.

[h] More details for quality control and quality criteria of MUD/SF medium are given in ISO 7899-1:1998, Annex E.

[i] Some national restrictions and directions may require the use of a different serovar. Make reference to national requirements relating to the choice of *Salmonella* serovars.

[j] Growth/turbidity is categorized as: 0 — no growth/turbidity; 1 — weak growth/turbidity; 2 — good growth/turbidity (see 7.4.1.2, 8.4.1).

[k] If BPW is used for two of these different applications: perform the *Salmonella* enrichment test as a minimum (if laboratory tests for this organism).

[l] Choose the strain(s) according to the method for which TSA is used as a reference medium.

Table F.2 — Abbreviated terms for media used in Table F.1

Media abbreviated term	Full name of the media	International Standard
BCYE	Buffered charcoal yeast extract agar medium	ISO 11731 and ISO 11731-2
Bolton	Bolton broth	ISO 17995
BPW	Buffered peptone water	ISO 6887 ISO 19250
DRCM	Differential reinforced clostridial medium	ISO 6461-1
GVPC	Buffered charcoal yeast extract agar with glycine, vancomycin, polymyxin B, cycloheximide	ISO 11731 and ISO 11731-2
Lactose TTC	Lactose triphenyltetrazolium chloride agar with sodium heptadecylsulfate	ISO 9308-1
mCCDA	Modified charcoal cefoperazone deoxycholate agar	ISO 17995
mCP	Membrane clostridium perfringens agar	Council Directive 98/83/EC
MUD/SF	4-methylumbelliferyl-α-D glucoside /SF medium	ISO 7899-1
MUG/EC	4-methylumbelliferyl-β-D glucuronide /EC medium	ISO 9308-3
Preston	Preston broth	ISO 17995
Pseudomonas CN	Pseudomonas cetrimide nalidixic acid agar	ISO 16266
RVS	Rappaport-Vassiliadis soya peptone broth	ISO 19250
Slanetz and Bartley	Slanetz and Bartley medium	ISO 7899-2
Sulfite Iron	Iron Sulfite agar	ISO 6461-2
Tryptose Sulfite (TS)	Tryptose sulphite agar	ISO 6461-2
TSA	Tryptone soya agar	—
TSC	Tryptose sulphite cycloserine agar (without egg yolk)	ISO 14189
XLD	Xylose lysine deoxycholate agar	ISO 19250
YEA	Yeast extract agar	ISO 6222

Annex G
(normative)

Use of control charts to monitor quantitative testing of solid culture media

G.1 General

This annex describes the use of control charts for monitoring results, particularly those expressed as a productivity ratio, P_R, as detailed in 7.2, when testing selective or non-selective agars against suitable non-selective reference agars or previously accepted batches of the same selective agar.

Care shall be taken when using control charts for batch-to-batch tests of the same agar, as any deterioration in the quality of successive batches might not be apparent unless test suspensions are carefully controlled to give consistent numbers of organisms, or RMs (see 3.4.6) are used. Any media QC system based on batch-to-batch checks shall be verified as fit for purpose before being brought into use.

Each source and batch of a test agar is likely to show different levels of productivity and individual laboratories can use different reference agars for comparison with the test agars. Therefore, individual laboratories shall set and justify their own limits and/or ranges for acceptable productivity ratios for each test agar in routine use within the range of productivity ratio values specified in 7.2.2.1.2. Control charts are prepared from initial validation data, acceptable limits set using statistical analysis and then the charts are used to monitor subsequent batches of agars. The acceptable limits are reviewed periodically (e.g. after every 30 tests) and the limits adjusted as necessary (more information is given in G.2.6).

The procedures require the use of a microbial suspension with a known concentration of the target strain specified for the test medium in Annex E or Annex F; this is the target value for the test. The test suspension should be either a commercial RM (see 3.4.6) or prepared by the laboratory from carefully standardized working cultures of reference strains. The concentration of organisms in the laboratory suspension (target value) shall be shown to be stable and homogeneous during the period of use.

To optimize control, such suspensions shall contain approximately 100 cfu (range 80 cfu to 120 cfu) in the volume of inoculum to be applied to the agar plates and the testing should normally be performed at least in duplicate. Table 1 (see5.4.2.5.1) gives the precision values at different levels of inoculation to show the importance of maintaining this optimal inoculum.

Agar plates shall be inoculated by the spread, pour or membrane filtration techniques, as appropriate to the reference standard method for which the agar is used, and incubated under the conditions defined in the individual standards.

Colonies present on or in each plate shall be counted in accordance with reference standard methods and productivity ratios calculated as in 7.2.1.1.

G.2 Using control charts

G.2.1 General procedure

To set up a new control chart, at least 10 tests on different batches of the same test agar shall be performed, preferably in duplicate, and on different days and by different technicians (intralaboratory reproducibility conditions). Using 20 tests (as shown in the procedure in G.2.2) will give more reliable limits so a chart prepared using 10 initial tests (the minimum number) shall be recalculated when

20 results are available. Individual charts shall be maintained on a rolling basis with at least 30 tests retained for each rolling assessment of ongoing media quality (see G.2.6).

G.2.2 Production of a control chart

This procedure is based on a standard inoculum containing (100 ± 20) cfu in the 0,1 ml inoculum used for a test carried out using the spread plate technique. It is also suitable where other inoculum volumes are used for other plating techniques, such as pour plate or membrane filtration, provided the inoculum contains a number of colonies within the acceptable range of (100 ± 20) cfu.

The average of the two sets of duplicate counts on test occasion i is x_i cfu/0,1 ml for the test medium and y_i cfu/0,1 ml for the reference medium. Then, the productivity ratio on occasion i is $x_i/y_i = r_i$. For a series of tests from i = 1, 2, …., n, where n is a minimum of 10 and preferably 20, use Formula (G.1) to calculate the mean P_R value $\bar{r}$:

$$\bar{r} = \frac{1}{n}\sum_{i=1}^{n} r_i = \frac{r_1 + r_2 + r_3 + \ldots r_n}{n} \quad \text{(G.1)}$$

Then, use Formula (G.2) to determine the mean range, $\bar{R}$, of the P_R values:

$$\bar{R} = \frac{1}{(n-1)}\sum_{i=2}^{n} |r_i - r_{i-1}| \quad \text{(G.2)}$$

where

i is the test number;

n is the total number of tests;

r_i is the the ith P_R value.

Then determine the standard deviation (s) as:

$$s = 0{,}886\ 5 \times \bar{R} = \frac{\bar{R}}{1{,}128} \quad \text{(G.3)}$$

NOTE The constant 0,886 5 (or its reciprocal 1,128) is the standard recommended by ASTM to determine standard deviation from the mean range of duplicate test values.

Calculate the 95 % (±2s) and the 99 % (±3s) limits of the distribution of the results.

On the Y-axis of the control, chart mark the positions of the overall mean P_R value, $\bar{r}$, and each of the two upper (+2s and +3s) and the two lower (–2s and –3 s) limits; then draw lines parallel to the X-axis. Plot the result of each individual P_R value for i = 1, 2, …. n on the X-axis (see the example).

Each time a new batch of medium is prepared, it is tested using the standard inoculum suspension and the P_R is calculated from the ratio of the number of colony-forming units counted after incubation on the test and reference media, as described above. This value is plotted on the control chart and checked against the derived limits.

G.2.3 Worked example of producing a control chart using 20 results

Table G.1 shows the results from 20 successive productivity ratio checks on batches of the same non-selective test agar using a standard inoculum of 110 cfu/0,1 ml counted on the reference non-selective medium (actual counts on the reference medium will vary from this figure in practice, but the data are used to show the principle of deriving the control chart from the calculated productivity ratios only).

Table G.1 — Record of 20 successive productivity ratio checks on a non-selective agar used to set up a control chart (showing actual counts on the test agar only; counts on reference medium y_i are 110 cfu throughout)

	Result of test number (i)									
i =	1	2	3	4	5	6	7	8	9	10
cfu (test agar - x_i)	95	102	94	97	105	68	98	105	103	116
r_i	0,86	0,93	0,85	0,88	0,95	0,62	0,89	0,95	0,94	1,05
$\lvert r_i - r_{i-1}\rvert$	–	0,07	0,08	0,03	0,07	–	0,06	0,06	0,01	0,11
i =	11	12	13	14	15	16	17	18	19	20
cfu (test agar - x_i)	95	90	89	116	114	110	114	98	88	102
r_i	0,86	0,82	0,81	1,05	1,04	1,00	1,04	0,89	0,80	0,93
$\lvert r_i - r_{i-1}\rvert$	0,19	0,04	0,01	0,24	0,01	0,04	0,04	0,15	0,09	0,13

It is important to note that the low result of test number 6 in this example for a non-selective agar was below the permitted range set for the acceptable P_R value of 0,70 to 1,40 for non-selective agar (see 7.2.2.1.2) and that all such results are excluded as 'outliers' in deriving the mean and standard deviation for the control chart.

The reasons for PR test results which fall outside the permitted range should be investigated as these are often associated with poor performance of the test rather than poor media quality, and are frequently found when the inoculum used is outside the specified precision range of 80 cfu to 120 cfu.

The mean P_R value is equal to:

$$\bar{r} = \sum r_i / n = (0{,}86 + 0{,}93 + 0{,}85 + \ldots + 0{,}80 + 0{,}93) / 19 = 17{,}5 / 19 = 0{,}92 \quad \text{(G.4)}$$

(shown by the solid line in Figure G.1).

The range values $\left(R = \lvert r_i - r_{i-1}\rvert\right)$ are determined as the absolute differences of sequential values, except in the case of an excluded value (e.g. test number 6 in Table G.1 above), i.e.:

0,93 – 0,86 = 0,07, 0,85 – 0,93 = 0,08, 0,88 – 0,85 = 0,03, etc.

The mean range is equal to:

$$\bar{R} = \sum R_i / (n-1) = (0{,}07 + 0{,}08 + 0{,}03 + \ldots + 0{,}09 + 0{,}13) / 18 = 1{,}43 / 18 = 0{,}08 \quad \text{(G.5)}$$

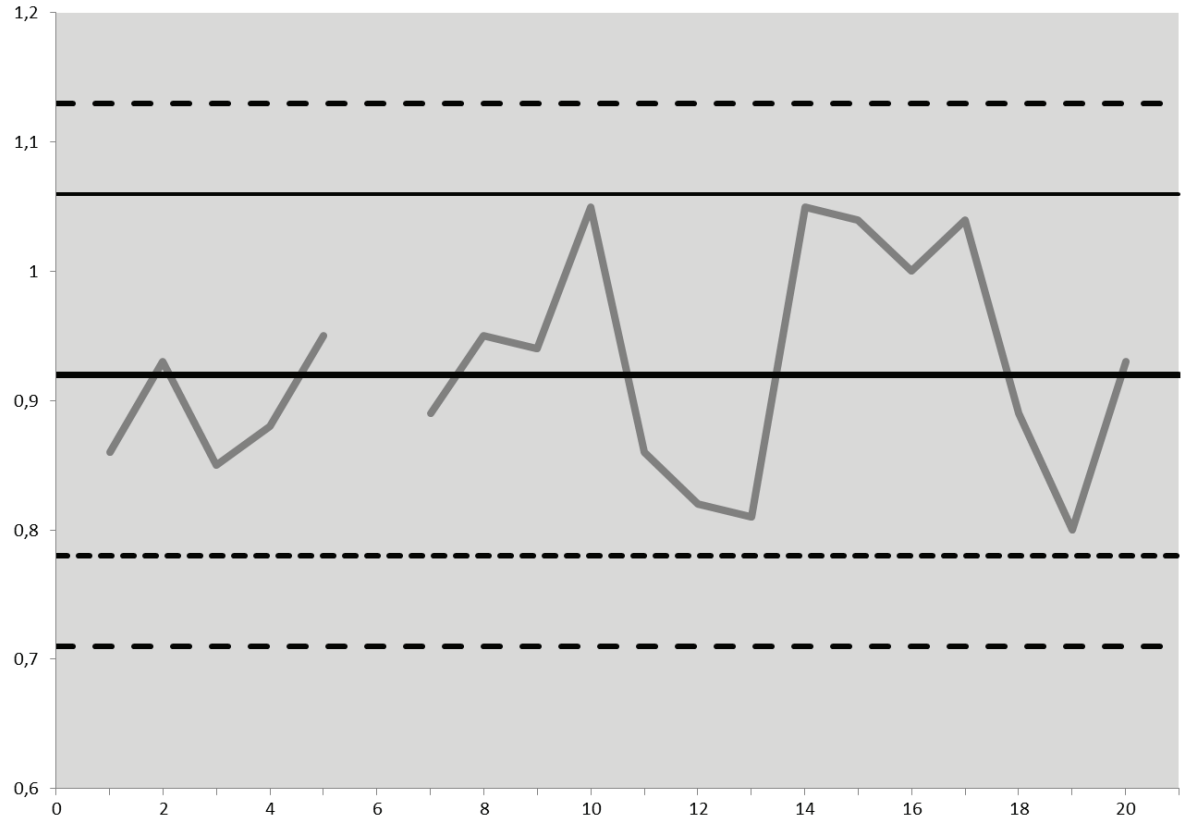
Thus, the standard deviation, s = 0,8865 × 0,08 = 0,071.

The 95 % confidence limits (shown by the light dotted lines in Figure G.1) are:

$$0{,}92 \pm 2 \times 0{,}071 = 0{,}92 \pm 0{,}14 = 0{,}78 \text{ to } 1{,}06 \quad \text{(G.6)}$$

The 99 % confidence limits (shown by the heavy dotted lines in Figure G.1) are:

$$0{,}92 \pm 3 \times 0{,}071 = 0{,}92 \pm 0{,}21 = 0{,}71 \text{ to } 1{,}13 \quad \text{(G.7)}$$



Key

Y productivity ratio, P_R

X test number

Figure G.1 — Control chart set up from the productivity ratios obtained from the first 20 checks shown in Table G.1 (outlier result 6 has been excluded)

G.2.4 Performance evaluation and interpretation of results

A batch of culture medium shall be accepted if both general (see 6.2) and microbiological quality criteria are met.

A new batch of media shall be rejected if any of the following results occur from quantitative tests performed as described above as these are "out of control" situations:

— a single violation of the ±3*s* action limit;

— two out of three observations in a row exceed the ±2*s* warning limit;

— six observations in a row that are steadily increasing or decreasing;

— nine observations in a row on the same side of the mean.

NOTE A criterion of four observations in a row exceeding a ±1 *s* level can also be useful as an indication of a developing problem.

G.2.5 Alternative approaches to monitoring media performance

The procedure for using a control chart to monitor media performance given above is specifically derived for plotting P_R values without $\log_{10}$ transformation of the colony count.

Alternative approaches are acceptable based on direct plotting of colony count values or $\log_{10}$-transformed colony counts, if these are shown to be appropriate. Methods to check whether colony count data distributions conform to normality or not without $\log_{10}$ transformation using the Kolmogorov-Smirnov test or the Chi-squared test, and worked examples of these, are given in NEN 6603.[39]

However, it should be noted that if the counts do not include the dilution factor then it is unlikely that a $\log_{10}$ transformation will be appropriate. Conversion of direct counts (e.g. 100 cfu/plate) should assume Poisson distribution for which the transformation of the count x is $\sqrt{x}$. Furthermore, in any situation where colony counts are plotted directly it is essential to ensure that the level of test inoculum is constant between tests, otherwise misleading results will be obtained.

G.2.6 Periodic review of control charts

The first and subsequent control charts for a single test medium shall be reviewed periodically to ensure that the limits set remain acceptable however the charts are derived. The first chart containing the minimum of 20 data points is reviewed to set the initial limits and then subsequent charts may be reviewed at the suggested frequency of every 30 data points by the procedures given below (in this subclause).

Once a control chart is complete, calculate the mean and standard deviation s again, omitting any results outside the acceptable range or limits set. Where a $\log_{10}$ transformation has been used, all calculations shall be carried out using the $\log_{10}$-transformed results.

Compare the standard deviation of the current chart with that of all previous results, s_{tot}, and check for variation using the following criterion:

$$\frac{s^2}{s_{tot}^2} < F(0{,}975;\ n-1, n_{tot}-1) \quad \text{(G.8)}$$

Where, F (0,975; n − 1, n_{tot} − 1) is the value of the F test at a probability of α = 0,025 at n observations of s, and n_{tot} observations of s_{tot}.

The variation is significantly increased where s does not fulfil this criterion and an investigation of the probable cause should be carried out.

Where the standard deviation of the resulting control chart fulfils the criterion, combine, for the next control chart, the data with the previous observations [see Formula (G.9) for an example of the calculations].

Also compare the mean, $\bar{x}$, of the current chart with the mean of all previous observations, $\bar{x}_{tot}$, and test using the following criterion:

$$\left|\bar{x} - \bar{x}_{tot}\right| < 2\sqrt{\frac{s^2}{n} + \frac{s_{tot}^2}{n_{tot}}} \quad \text{(G.9)}$$

where

$\bar{x}$	is the mean value of the current control chart;
$\bar{x}_{tot}$	is the mean value of all previous control charts;
s	is the standard deviation of the control chart;
s_{tot}	is the standard deviation of all previous control charts;
n	is the total number of observations of the current control chart;
n_{tot}	is the total number of observations of all previous control charts.

If the mean of the current control chart fulfils the criterion, combine the data with the previous observations for the next control chart.

Start an investigation into the probable cause if the criterion is not fulfilled. If the cause cannot be elucidated, start a new control chart and set new limits based on previous observations by recalculating the limits from the mean and standard deviation of all observations.

EXAMPLE Example of calculation:

Examples of data are given in Table G.2, based on three control charts each containing 30 observations. x_{tot}, s_{tot} and s^2_{tot} relate to combined data from Charts 1 and 2.

One of the observations in the second chart (number 22, marked in italics) exceeds the acceptable limit set by the laboratory from their own data and this observation is therefore not used in the calculations.

Table G.2 — Data for 3 control charts for evaluation against limits set by a laboratory (from NEN 6603[39])

	Test results									
Measurement	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Chart 1	100	77	108	75	83	92	70	81	90	88
Chart 2	74	81	60	66	109	83	73	82	74	89
Chart 3	89	75	67	63	90	77	90	75	53	91
Measurement	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Chart 1	78	82	90	95	75	86	100	98	75	92
Chart 2	80	83	95	71	98	74	76	92	84	88
Chart 3	99	74	88	68	100	81	97	89	80	71
Measurement	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Chart 1	74	80	98	79	82	91	78	90	65	60
Chart 2	67	*130*	97	98	64	85	101	73	67	82
Chart 3	63	84	82	84	99	79	86	70	72	98
Calculations for individual chart data										
	$\bar{x}$	s	s^2			x_{tot}	s_{tot}	s^2_{tot}	**From combined data for Charts 1 and 2**	
Chart 1	84,4	11,1	122,7			83,0	11,7	136,1		
Chart 2	81,6	12,3	150,8			–	–	–		
Chart 3	81,1	12,1	147,5			–	–	–		

Testing for variation in the standard deviation by comparing the standard deviation, s, of the latest chart (Chart 3) to that of the first two charts ($s$tot) as specified above gives the following result:

$$\frac{s^2}{s^2_{tot}} = \frac{147,5}{136,1} = 1,084 \qquad \text{(G.10)}$$

The critical value is F (0,975; 29,58) = 1,83. The observed value is smaller than the critical value so there is no significant difference between the standard deviation from Chart 3 and the previous charts (Charts 1 and 2).

Then testing if the mean value has changed as described above for these data gives the following result:

The data for Chart 3 gives |81,1 − 83,0| = 1,9 and the critical value gives:

$$2\sqrt{\frac{147,5}{30}+\frac{136,1}{59}}=5,4 \tag{G.11}$$

The calculated value for Chart 3 is smaller than the critical value so there is no significant difference between the mean of Chart 3 and the previous charts (Charts 1 and 2).

As the test for variation was also valid, the data from Chart 3 are combined with the previous charts for calculation of the new limits. Thus, the new chart is made on the basis of a mean value of 82,4 and a standard deviation of 11,80.

Annex H
(informative)

Quality assurance of culture media — Troubleshooting

Table H.1

Abnormality	Possible reason
Agar medium fails to solidify	Overheating of medium during preparation Low pH causing acid hydrolysis to occur Incorrect mass of agar used Agar not thoroughly dissolved Poor mixing of ingredients
Incorrect pH	Overheating of medium during preparation Poor water quality Extraneous chemical contamination pH measured at incorrect temperature pH meter incorrectly calibrated Poor quality dehydrated medium
Abnormal colour	Overheating of medium during preparation Poor water quality Poor quality dehydrated medium Absence of one or more ingredients Incorrect ingredients used Incorrect pH Extraneous contamination
Formation of precipitates	Overheating of medium during preparation Poor water quality Poor quality dehydrated medium Poor pH control If preparing from individual components – impurities in raw materials
Medium inhibitory/Low productivity	Overheating of medium during preparation Poor quality dehydrated medium Poor water quality Incorrect formulation used, e.g. ingredients not weighed out correctly, supplements added at wrong concentration Toxic residues present in preparation vessels or water Control organism(s) incorrectly prepared

Table H.1

Abnormality	Possible reason
Poor selectivity/specificity	Overheating of medium during preparation
	Poor quality dehydrated medium
	Incorrect formulation used
	Supplements added incorrectly e.g. when medium too hot or at wrong concentration
	Supplement contaminated
	Control organism(s) incorrectly prepared
Contamination	Improper sterilization
	Poor aseptic techniques
	Supplement contaminated

Annex I
(informative)

Quantitative testing of liquid media

I.1 General

This annex contains methods for quantitative testing of liquid media, which may provide additional information compared with the routine methods described in Clause 8. The methods are mainly applicable to evaluation of media under development and for comparative studies.

The quality of a liquid medium with respect to optimal growth properties is indicated most appropriately in the early growth phase. Looking at the length of the lag phase and growth in the early log phase gives the most sensitive information on productivity and selectivity of target and non-target microorganisms respectively in the test and reference broths. Therefore if only minor differences in the quality are being sought, streaking from the liquid medium onto the plates should be done after a shorter incubation period of e.g. Six h or 12 h.

I.2 Method for quantitative testing of non-selective liquid culture media using target microorganisms

I.2.1 Procedure

— Select a number of tubes each containing at least 10 ml of medium or 10 ml portions from each batch to be tested (see 3.1.2).

— Use working cultures as described in 5.4.2.2.

— Inoculation of target microorganisms: Inoculate test broth and solid reference medium for each test organism with ≤ 100 cells.

— Incubate the inoculated media under the conditions defined in the individual standards.

— Prepare sufficient dilutions from the incubated media to achieve a countable number of colonies (see 5.4.2.5).

— Spread a measured volume, e.g. 10 µl, on a non-inhibitory agar plate as described in 7.2.2.1.1.

I.2.2 Counting and interpretation of results

After incubation, count the number of colonies on the plates (see 7.2.2.1.1 and 7.2.2.1.2). Interpretation of the results will depend on the objective of the test, e.g. comparison with previous batch, reference medium or RM.

Target microorganisms should reach 10^6 cfu/ml to 10^8 cfu/ml.

I.2.3 Flow chart for quantitative method for non-selective liquid culture media using target microorganisms

Figure I.1 is the flow chart for quantitative method for non-selective liquid culture media using target microorganisms.

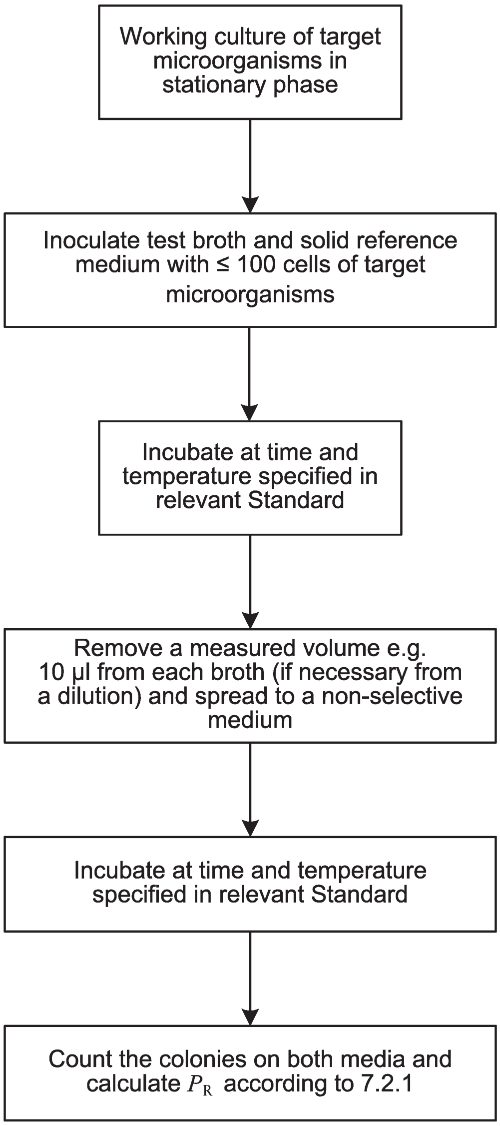


Figure I.1 — Flow chart for performance testing of non-selective liquid culture media using target microorganisms (see I.2.1 and I.2.2)

I.3 Method for quantitative testing of selective liquid culture media using target and non-target microorganisms

I.3.1 Procedure

— Select a number of tubes each containing at least 10 ml of medium or 10 ml portions from each batch to be tested (see 3.2.2).

— Use working cultures as described in 5.4.2.

— Inoculation of target microorganisms: inoculate test broth, reference broth and solid reference medium for each test organism with ≤100 cells. The solid reference medium is used to enumerate the cfu in the inoculum.

— Inoculation of non-target microorganisms: inoculate test broth, reference broth and solid reference medium for each test organism with ≥1 000 cells.

— Inoculation of target and non-target microorganisms as a mixed culture: For testing mixed cultures in selective media inoculate test broth, reference medium and solid reference medium with ≤ 100 cells of target microorganism and ≥1000 cells of non-target microorganisms in the same tube/on the same plate. To test mixed cultures, spreading should be done when possible on non-selective agar plates which allow differentiation of the microorganisms in the mixed culture (e.g. plate count agar with MUG for counting *Escherichia coli* and *Salmonella* spp.). When it is not possible to distinguish mixed cultures on non-selective agar, selective agar media should be used provided their performance has been previously tested.

— Incubate the media under the conditions defined in the individual standards.

Remove a measured volume or if necessary a volume after dilution from each broth and spread on a non-inhibitory agar plate for tubes containing only target microorganisms or only non-target microorganisms as described in 8.2.2. For tubes containing both target and non-target microorganisms, spread on a plate of the same selective medium used above.

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

I.3.2 Reading, calculation and interpretation of results

Count the colonies of target and non-target microorganisms on each plate and calculate the recovery compared with the reference broth, taking into account the dilution used for counting (if needed). In the case of mixed cultures, distinguish the different types.

Calculation and interpretation depends on the aim of examination. Interpretation of the results will depend on the objective of the test, e.g. comparison with previous batch, reference medium or RM.

The target microorganism should reach 10^6 cfu/ml to 10^8/ml and should be the dominant organism present in selective media.

For mixed cultures, the recovery of the target organism should not be reduced compared with the recovery from the pure culture of target organism.

I.3.3 Flow chart for quantitative method for selective liquid culture media using target and non-target microorganisms

Figure I.2 is the flow chart for quantitative method for selective liquid culture media using target and non-target microorganisms

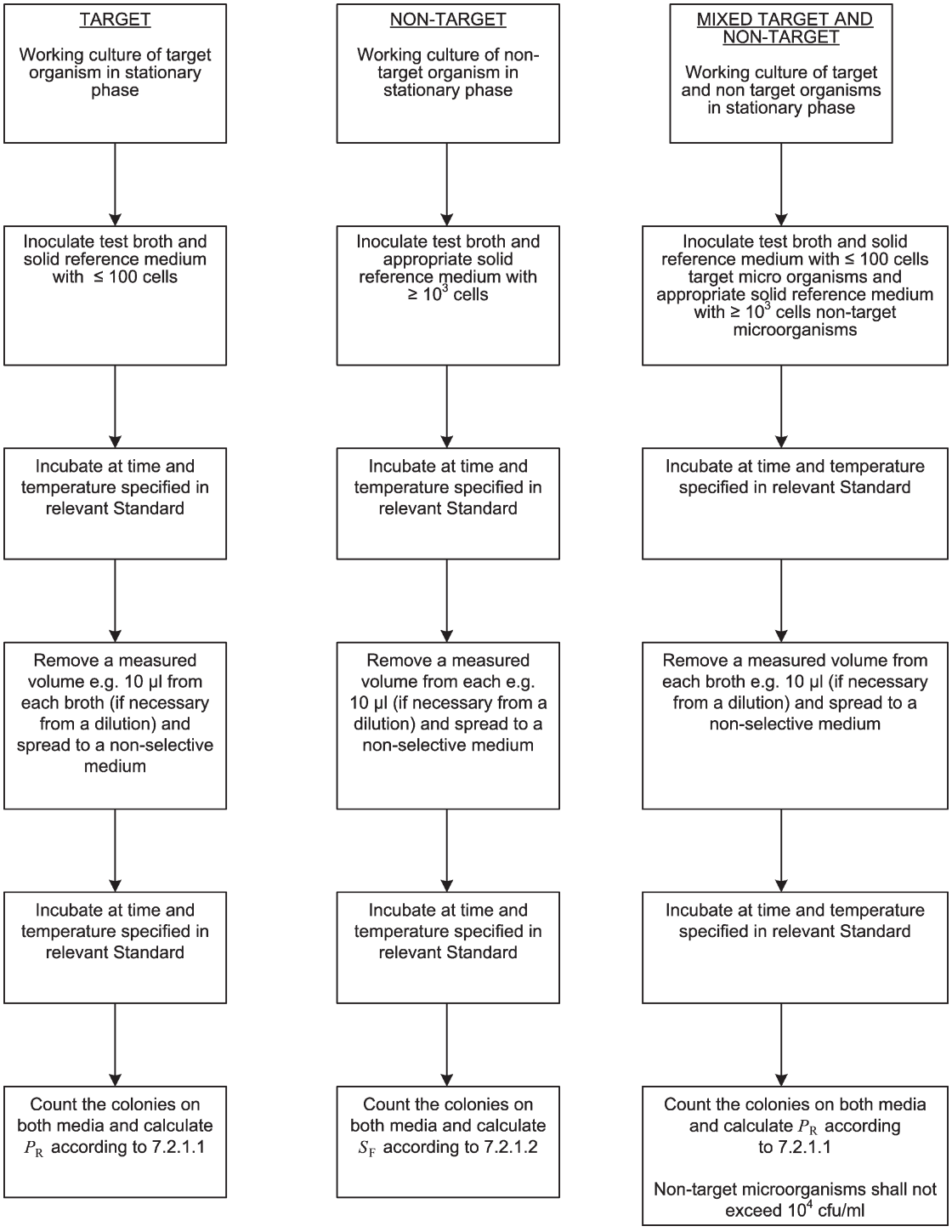


Figure I.2 — Flow chart for quantitative testing of selective liquid culture media using target and non-target microorganisms (see I.3.1 and I.3.2)

Annex J
(normative)

Definition of microbiological performance tests for standardized culture media

J.1 General

This annex provides project leaders of standardization working groups with instructions to enable them to define microbiological performance criteria, methods and control strains, when creating or revising International Standards on microbiological analysis (food microbiology or microbiology of water).

J.2 General

Microbiological performance requirements (performance criteria, control method and targets) applicable to standardized culture media shall be included in each International Standard on microbiological analysis.

These performance specifications may be:

— either imported, and revised if necessary, from this International Standard for the culture media described therein;

— or created, for any new culture medium, in accordance with the principles defined in this annex.

NOTE This definition of microbiological performance requirements does not address reagents or media for confirmation.

J.3 Performance criteria, methods and targets

The criteria under evaluation (productivity, selectivity, specificity), the test methods to be used (quantitative and qualitative) and the targets to be met shall be defined according to the characteristics of each culture medium, as shown in Table J.1 below:

— its form (broth, agar);

— its composition (selective, non-selective medium);

— its function in the standardized method (enrichment, dilution, detection, enumeration).

Table J.1 — Criteria, methods and targets

Medium	Criterion and test method
Selective enumeration broth	Productivity (method: quantitative)[e] Selectivity (qualitative)[f]
Selective enumeration agar	Productivity (quantitative)[b] Selectivity (qualitative)[g] Specificity (qualitative)[i]
Selective enrichment broth	Productivity (qualitative)[c] Selectivity (qualitative)[h]

Table J.1 *(continued)*

Medium	Criterion and test method
Selective detection agar	Productivity (qualitative)[d] Selectivity (qualitative)[g] Specificity (qualitative)[i]
Non-selective enumeration agar	Productivity (quantitative)[b]
Non-selective enrichment broth	Productivity (qualitative)[a]
Non-selective dilution broth	Productivity (quantitative)[e]
Non-selective detection agar	Productivity (qualitative)[d]

1. Productivity: the purpose of this performance criterion is to verify the satisfactory growth of the target strains (and the colony morphology), on agar media.

[a] Qualitative productivity (liquid medium): see 8.4.

Target: the result shall be 2 (satisfactory growth) for an inoculum of ≤ 100 cfu of the target microorganisms (8.4.1).

[b] Quantitative productivity (agar medium): see 7.2.1.1.

— Target: the P_R shall be ≥ 0,50 for comparison of a selective medium with the non-selective reference medium specified in Annexes E and F. The P_R shall be ≥ 0,70 for comparison of a non-selective medium with a non-selective reference medium or as specified in the standard or Annexes E and F. This also applies to special cases where comparison is made with the previous batch.

The target microorganism colonies shall have a characteristic appearance.

[c] Qualitative (liquid medium): see 8.3.

Target: At least 10 colonies of the target microorganism shall be observed on the selective isolation medium used for detection after inoculation with ≤ 100 cfu.

[d] Qualitative productivity (agar medium): see 7.4.

Target: the result shall be 2 (satisfactory growth) for the target microorganisms.

The target microorganism colonies shall have a characteristic appearance.

[e] Quantitative productivity (liquid medium): see 8.2.

Target: For diluents, the number of microorganisms, after the contact time, shall be within ± 30 % of the initial number.

2. Selectivity: the purpose of this performance criterion is to verify that interfering (non-target) strains are either partially or totally inhibited by the selective medium.

[f] Qualitative selectivity (liquid medium): see 8.3.

Target: the tested (undesired) microorganisms shall be totally inhibited.

[g] Qualitative selectivity (agar medium): see 7.4.

Target: the tested (undesired) microorganisms shall be partially or totally inhibited.

[h] Qualitative selectivity (liquid medium): 8.3.

Target: No growth (or < 10 cfu) of the (undesired) microorganisms shall appear on the non-selective agar used for detection.

3. Specificity: the purpose of this performance criterion is to verify that the interfering (non-target) strains which are not inhibited do not produce characteristic colonies.

[i] Qualitative specificity (agar medium): 7.4.

Target: the colonies of the tested non-target strains shall not have the characteristic appearance of the target microorganisms in the standardized method.

The criteria shall be verified with standardized incubation times and temperatures.

J.4 Choice of performance control strains

J.4.1 General

The targets of the various performance criteria and the scope of the standardized method shall determine the choice of control strains, i.e.

— the target strains (productivity) detected or enumerated in the method, and

— the non-target strains (selectivity, specificity) belonging to the interfering flora commonly encountered in the matrices under analysis.

The control strains described in Annex E and Annex F, which have already been tested on a given medium, shall be used if possible.

If justified (genetic instability, insufficient reproducibility of previously defined QC strains, etc.), the project leader may propose a modification of the required strains, in accordance with the instructions in this subclause.

J.4.2 Assessment of suitability of new control strains

Before introduction of a new strain, its suitability for use in performance testing shall be verified and documented. This requires demonstration of the reproducibility of the performance control in at least two (preferably three) independent laboratories using two or three different lots of the standardized formula or from different commercial producers in each laboratory.

J.4.3 New media

For any new medium, the control strains shall be:

— preferentially selected from those already described in Annex E or Annex F;

— previously assessed and demonstrated to be suitable (see J.4.2);

— or newly introduced under the following conditions:

  — they shall be, preferentially, of food origin or be obtained from water samples;

  — they shall be available in at least two international strain collections (mutually equivalent), at an acceptable price;

  — they shall be previously assessed and demonstrated to be suitable (see J.4.2).

J.4.4 Number of strains per criterion

Table footnote a Qualitative productivity (liquid medium): see 8.4.

Two target strains: strain type A and strain type B

Type A: strain tested in performance quality control by medium manufacturer and end user

Type B: strain required to be tested only by medium manufacturer.

Table footnote b Quantitative productivity (agar medium): see 7.2.1.1.

Two target strains: strain A and strain B

Table footnote c Qualitative productivity (liquid medium): see 8.3.

Two target strains: strain A and strain B each associated with two non-target strains

Table footnote d Qualitative productivity (agar medium): see 7.4

Two target strains: strain A and strain B

Table footnote e Quantitative productivity (liquid medium): see 8.2

Two target strains: strain A and strain B

Table footnote f Qualitative selectivity (liquid medium): see 8.3

Two non-target strains: strain A and strain B

Table footnote g Qualitative selectivity (agar medium): see 7.4

Two non-target strains: strain A and strain B

Table footnote h Qualitative selectivity (liquid medium): 8.3

Two non-target strains: strain A and strain B

Table footnote i Qualitative selectivity (agar medium): 7.4

One non-target strain: strain A

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