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Microbiology of the food chain — Horizontal method for the detection and enumeration of *Enterobacteriaceae* —

Part 1: Detection of *Enterobacteriaceae*

Microbiologie de la chaîne alimentaire — Méthode horizontale par la recherche et le dénombrement des Enterobacteriaceae —

Partie 1: Recherche des Enterobacteriaceae

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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 World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html

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

This second edition cancels and replaces the first edition (ISO 21528-1:2004), which has been technically revised with the following main changes:

— the MPN method has become an informative Annex A;

— the pre-enrichment step in BPW followed by enrichment in EE broth has been changed to enrichment in BPW[7] and confirmation now takes place in Glucose OF medium instead of using glucose agar;

— performance testing for the quality assurance of the culture media has been added;

— performance characteristics for this method have been added to Annex C.

A list of all the parts in the ISO 21528 series can be found on the ISO website.

Introduction

This document is intended to provide general guidance for the examination of products not dealt with by existing International Standards and to be taken into account by organizations preparing microbiological test methods for application to foods or animal feeding stuffs. Because of the large variety of products within this field of application, these guidelines may not be appropriate in every detail for certain products, and for some other products it may be necessary to use different methods. Nevertheless, it is hoped that in all cases, every attempt will be made to apply the guidelines provided as far as possible and that deviations from them will only be made if absolutely necessary for technical reasons.

The main changes, listed in the foreword, introduced in this document compared to ISO 21528-1:2004 are considered as major (see ISO 17468).

The harmonization of test methods cannot be immediate, and for certain groups of products, International Standards and/or national standards may already exist that do not comply with this horizontal method. It is hoped that when such standards are reviewed, they will be changed to comply with this document so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

Microbiology of the food chain — Horizontal method for the detection and enumeration of *Enterobacteriaceae* —

Part 1: Detection of *Enterobacteriaceae*

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detecting *Enterobacteriaceae* are only undertaken in properly equipped laboratories under the control of a skilled microbiologist, and that great care is taken in the disposal of all incubated materials. Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety aspects, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices.

1 Scope

This document specifies a method, with enrichment, for the detection of *Enterobacteriaceae*. It is applicable to

— products intended for human consumption and the feeding of animals, and

— environmental samples in the area of primary production, food production and food handling.

This method is applicable

— when the microorganisms sought are expected to need resuscitation by enrichment, and

— when the number sought is expected to be below 100 per millilitre or per gram of test sample.

A limitation on the applicability of this document is imposed by the susceptibility of the method to a large degree of variability (see Clause 11).

NOTE Enumeration can be carried out by calculation of the most probable number (MPN) after incubation in liquid medium. See Annex A.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6887 (all parts), *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

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

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1
Enterobacteriaceae
microorganism that forms characteristic colonies on violet red bile glucose agar and that ferment glucose and show a negative oxidase reaction when the tests are carried out in accordance with the methods specified in this document

3.2
detection of *Enterobacteriaceae*
determination of *Enterobacteriaceae* (3.1), in a particular mass or volume of product or surface area, when tests are carried out in accordance with this document

4 Principle

4.1 Enrichment in non-selective medium

Buffered peptone water (BPW) is inoculated with the test portion, and then incubated at 37 °C (or 30 °C) for 18 h.

NOTE The incubation temperature of 37 °C for enrichment and isolation/enumeration on plating medium is generally used when the detection and enumeration of *Enterobacteriaceae* is for a hygiene indicator. Alternatively, a temperature of 30 °C can be chosen when the detection or enumeration of *Enterobacteriaceae* is conducted for technological purposes and includes psychrotrophic *Enterobacteriaceae*. In this document, 37 °C will be used throughout the text.

4.2 Isolation and selection for confirmation

Violet red bile glucose (VRBG) agar is inoculated with the culture obtained after enrichment in BPW, then incubated at 37 °C. It is examined after 24 h to detect the presence of typical colonies of presumptive *Enterobacteriaceae.*

4.3 Confirmation

Typical colonies of presumptive *Enterobacteriaceae* are subcultured onto non-selective medium, and confirmed by means of tests for the fermentation of glucose and the presence of oxidase.

5 Diluent, culture media and reagent

For current laboratory practice, see ISO 7218.

Composition of culture media and reagents and their preparation are specified in Annex B.

For performance testing of culture media, see ISO 11133 and Annex B.

6 Equipment and consumables

Disposable equipment is an acceptable alternative to reusable glassware if it has suitable specifications. Usual microbiological laboratory equipment (see ISO 7218) and, in particular, the following.

6.1 Apparatus for dry sterilization (oven) **or wet sterilization** (autoclave), as specified in ISO 7218.

6.2 Incubator, capable of operating at 37 °C ± 1 °C (or 30 °C ± 1 °C).

6.3 Drying cabinet (ventilated by convection) or **incubator**, capable of operating between 25 °C and 50 °C.

6.4 Water bath, or similar apparatus, capable of being maintained between 47 °C to 50 °C.

6.5 Containers (e.g. bottles, tubes, flasks), suitable for the sterilization and storage of culture media.

6.6 Test tubes or **flasks** of appropriate capacity.

6.7 Petri dishes, made of glass or plastics, of diameter 90 mm to 100 mm.

6.8 Loops (of diameter approximately 3 mm) and **wires**, made of platinum/iridium or nickel/chromium, or **glass rods**, or equivalent sterile disposable loops or inoculating needles.

6.9 Graduated pipettes or **automatic pipettes**, of nominal capacities 10 ml, 1 ml and 0,1 ml.

6.10 pH-meter, accurate to within ±0,1 pH unit at 25 °C.

6.11 Homogenizer, as specified in ISO 7218.

7 Sampling

Sampling is not part of the method specified in this document. See the specific International Standard dealing with the product concerned. If there is no specific International Standard dealing with the sampling of the product concerned, it is recommended that the parties concerned come to an agreement on this subject.

Recommended sampling techniques are given in:

— ISO/TS 17728 for food and animal feed;

— ISO 13307 for primary production stage;

— ISO 17604 for carcasses;

— ISO 18593 for environmental samples.

It is important that the laboratory receives a sample that is representative and the sample should not have been damaged or changed during transport or storage.

8 Preparation of test sample

Prepare the test sample in accordance with the specific International Standard appropriate to the product concerned. If there is no specific International Standard available, it is recommended that the parties concerned come to an agreement on this subject.

9 Procedure

9.1 General

See ISO 7218.

9.2 Test portion and initial suspension

In general, an amount of test portion (mass or volume) is added to a quantity of BPW (mass or volume) to yield a 10-fold dilution. For example, a 10 g test portion is mixed with 90 ml of BPW.

This document has been validated for test portions of 10 g or ml. A smaller test portion may be used, without the need for additional validation/verification, providing that the same ratio between enrichment broth and test portion is maintained. A larger test portion than that initially validated may be used, if a validation/verification study has shown that there are no adverse effects on the detection of *Enterobacteriaceae.*

NOTE Validation can be conducted in accordance with the appropriate documents of ISO 16140 (all parts). Verification for pooling samples can be conducted in accordance with the protocol described in ISO 6887-1:2017, Annex D.

9.3 Enrichment

Incubate the initial suspension (9.2) at 37 °C for 18 h ± 2 h.

Continue the procedure with isolation and selection of colonies for confirmation (9.4).

9.4 Isolation and selection for confirmation

9.4.1 Isolation

Using a loop (6.8), streak from the incubated enrichment medium (see 9.3) the surface of a plate containing the selective medium (B.2) and incubate the plate at 37 °C (see note in Clause 4) for 24 h ± 2 h.

9.4.2 Selection of colonies for confirmation

Characteristic colonies are pink to red or purple (with or without precipitation haloes).

Mark suspect colonies from the incubated plates (see 9.4.1). Select at least one typical or suspect colony for subculture (see 9.5) and biochemical confirmation tests (see 9.6). If this is negative, select up to four more suspect colonies.

If more than one morphology is present among the colonies, select one colony of each morphology for subculture.

Certain *Enterobacteriaceae* may cause decolouration of their colonies or of the medium. Therefore, when no characteristic colonies are present, choose whitish colonies for confirmation.

9.5 Subculturing selected colonies

Streak onto non-selective medium (e.g. nutrient agar plates) (B.3) each of the colonies selected for confirmation (see 9.4.2).

Incubate these plates at 37 °C for 24 h ± 2 h.

Select a well-isolated colony from each of the incubated plates for the biochemical confirmation tests (see 9.6).

9.6 Biochemical confirmation tests

9.6.1 Oxidase reaction

Using a platinum/iridium loop, wire or glass rod (6.8), take a portion of each well-isolated colony (see 9.5) and streak onto a filter paper moistened with the oxidase reagent (B.5) or onto a commercially available disc or stick. A nickel/chromium loop or wire shall not be used.

Consider the test to be negative if the colour of the filter paper does not turn dark blue purple within 10 s.

Consult the manufacturer's instructions for ready-to-use discs or sticks.

9.6.2 Fermentation test

Using a wire (6.8), stab the same colonies selected in 9.6 that gave a negative oxidase test into tubes containing Glucose OF medium (B.4). Overlay the surface of the medium with minimal 1 cm of sterile mineral oil (B.6).

Incubate these tubes at 37 °C for 24 h ± 2 h.

If a yellow colour develops throughout the content of the tube, regard the reaction as being positive.

10 Expression of results

If any of the selected typical colonies (see 9.4.2) from a subculture (see 9.4.1) is oxidase-negative and glucose-positive, the sample from which the subculture was derived shall be regarded as being positive for *Enterobacteriaceae*. In accordance with the interpretation of the results, indicate *Enterobacteriaceae* detected or not detected in a test portion of *x* g or *x* ml of product, or on the surface area swabbed, or in entire objects (e.g. boot socks).

11 Precision

11.1 Interlaboratory study

The performance characteristics of the method were determined in an interlaboratory study to determine the specificity, sensitivity and the LOD_{50} of the method. The data are summarized in Annex C. The values derived from the interlaboratory study may not be applicable to food types other than those given in Annex C.

NOTE In this document, the word "type" is combined with "food" to improve the readability of this document. However, the word "food" is interchangeable with "feed" and the other areas of the food chain as mentioned in the scope of this document.

11.2 Sensitivity

The sensitivity is defined as the number of samples found positive divided by the number of samples tested at a given level of contamination. The results are thus dependent on the level of contamination of the sample.

11.3 Specificity

The specificity is defined as the number of samples found negative divided by the number of true negative (or blank) samples tested.

12 Test report

The test report shall specify:

— the test method used, with a reference to this document, i.e. ISO 21528–1;

— the sampling method used, if known;

— the size of the test portion and/or the nature of the objects examined (for detection methods only);

— all operating conditions not specified in this document, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

— any deviations (e.g. in the media or the incubation conditions used);

— all information necessary for the complete identification of the sample;

— the test result(s) obtained.

13 Quality assurance

The laboratory should have a clearly defined quality control system to ensure that the equipment, reagents and techniques are suitable for the test. The use of positive controls, negative controls and blanks are part of the test. Performance testing of culture media is specified in Annex B and described in ISO 11133.

Annex A
(informative)

Enumeration by MPN technique

A.1 General

The most probable number (MPN) technique can be used when the number of *Enterobacteriaceae* sought is expected to be in the range 1 to 100 per millilitre or per gram of test sample. The MPN technique follows the same principles as the detection method, but several dilutions are tested. It is known that wide variations in results can occur using this technique for enumeration. The MPN technique can however be used when low numbers are expected or because of the nature of the product under test, according to the instructions in this annex.

A.2 Principle

A.2.1 Enrichment

A test portion of x g is added to 9 × x ml of buffered peptone water (BPW) and homogenized. One or more 10-fold dilutions (according to the expected level of contamination) are prepared in BPW. Aliquots (10 ml) of this initial dilution are transferred to three tubes. Then 3 × 1 ml of the initial dilution are added to 9 ml of BPW and 3 × 1 ml of each further dilution are added to 9 ml of BPW. These tubes are incubated 37 °C for 18 h ± 2 h.

A.2.2 Isolation and selection for confirmation

A selective solid medium (violet red bile glucose agar) is inoculated with a loop from each of the incubated cultures obtained after enrichment in BPW, then incubated at 37 °C (or 30 °C, see note in Clause 4). It is examined after 24 h ± 2 h to detect for the presence of presumptive *Enterobacteriaceae* colonies.

A.2.3 Confirmation

Colonies of presumptive *Enterobacteriaceae* are subcultured on a non-selective medium and then confirmed by means of tests for the fermentation of glucose and the presence of oxidase.

A.2.4 Calculation

The most probable number of *Enterobacteriaceae* per millilitre or per gram of the test sample is calculated from the number of confirmed positive tubes using a MPN table (see ISO 7218).

A.3 Procedure (see Figure A.1)

A.3.1 Test portion, initial suspension and further dilutions

Depending on the desired accuracy of the results, inoculate an appropriate number of flasks or tubes with the same dilution (e.g. three, five or 10 flasks or tubes). As a general rule, the techniques specified require three flasks or tubes per dilution.

For preparation of the initial suspensions and further dilutions, use the enrichment medium (B.1).

To prepare the initial suspension, take a test portion of x g or x ml and homogenize in 9 × x ml of buffered peptone water (BPW) (B.1). A 10^{-1} dilution is thus obtained.

NOTE 10 ml of the 10^{-1} dilution contain the equivalent of 1 g or 1 ml of sample.

Prepare further dilutions by taking 1 ml of the 10^{-1} dilution and add to 9 ml of BPW. Repeat to make further necessary decimal dilutions.

Transfer three 10 ml volumes of the 10^{-1} suspension to tubes (6.6) and three 1 ml volumes of the 10^{-1} dilution to tubes containing 9 ml of BPW (B.1).

A.3.2 Enrichment

Incubate the initial suspensions and dilutions (total 9 tubes) (see 9.4.1) at 37 °C for 18 h ± 2 h.

A.3.3 Isolation, subculturing and confirmation

See 9.4, 9.5 and 9.6.

A.4 Expression of results

Count the number of tubes giving a positive confirmed reaction for each dilution. Calculate the MPN from the number of positive confirmed tubes at each dilution.

For the calculation of the MPN, the formulas given in ISO 7218 can be used. As no "standard" MPN tables are known to be available for the dilutions used, a software program for use in Excel®[1)] has been written that can handle up to 10 levels of serial dilutions. It is highly recommended that this program be used rather than other programs, since the results for any specific combination of results derived with three dilutions will be the same as those in the tables published in ISO 7218. Details of the calculations are described in Reference [7] and the software is freely available for download from:

http://standards.iso.org/iso/7218

A.5 Precision

It is known that wide variations in results can occur with the MPN technique. Results obtained by this method should therefore be used with caution. Confidence limits are given in ISO 7218.

1) Excel® is the trademark of product supplied by Microsoft. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or of the product named. Equivalent products may be used if they can be shown to lead to the same results.

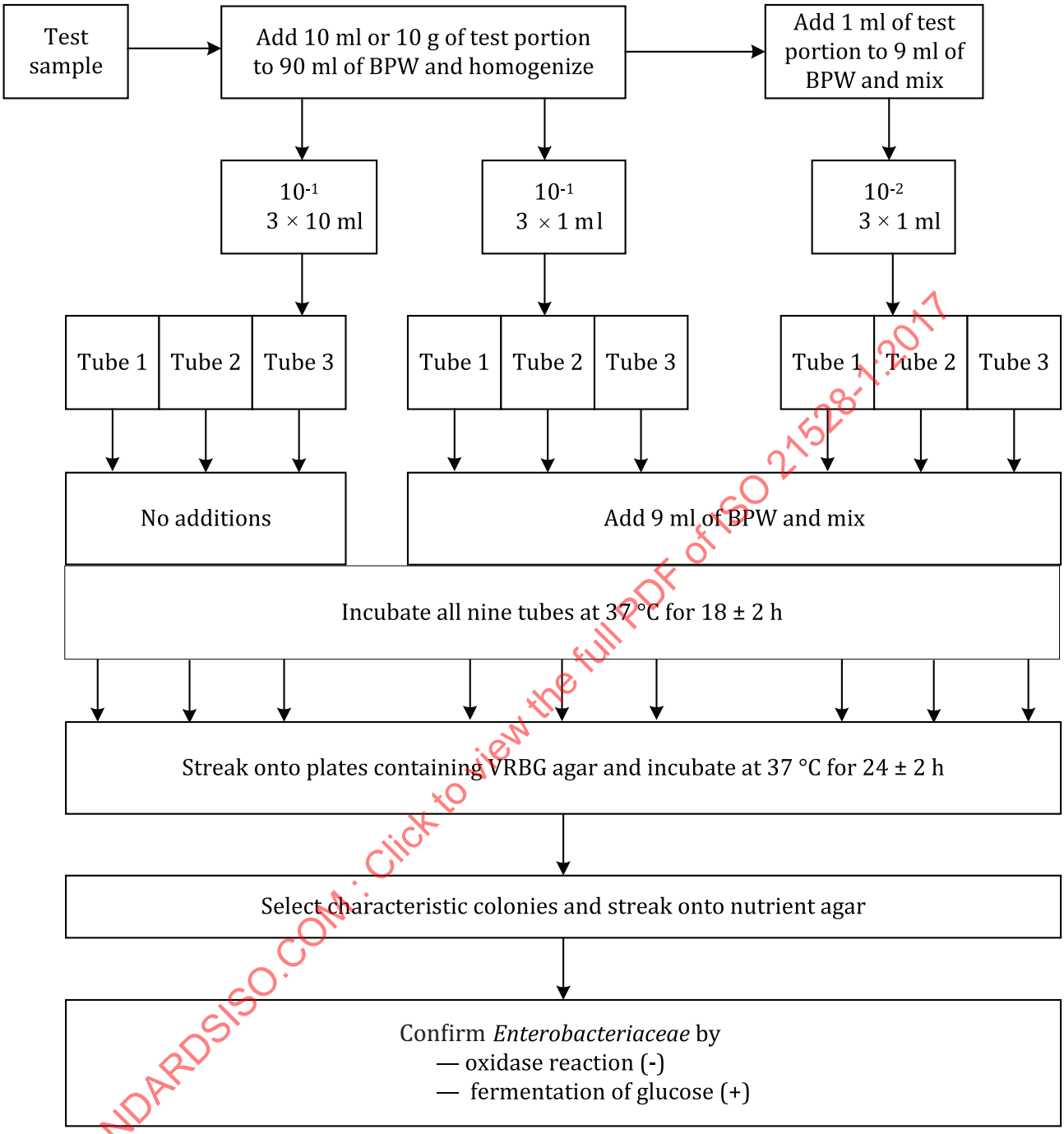

Figure A.1 — Diagram of procedure by MPN technique

Annex B
(normative)

Culture media and reagents

B.1 Enrichment medium and diluent: buffered peptone water (BPW)

B.1.1 General

BPW is used as the enrichment medium for the detection and enumeration method. See ISO 18593 for neutralising diluents when using swab samples.

B.1.2 Composition

Peptone[a]	10,0 g
Sodium chloride	5,0 g
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4.12H_2O$)	9,0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1,5 g
Water	1 000 ml

[a] For example enzyme digest of casein.

B.1.3 Preparation

Dissolve the components in the water, by heating if necessary.

Adjust the pH, if necessary, so that after sterilization it is 7,0 ± 0,2 at 25 °C.

Dispense the medium into sterile flasks or tubes (6.5) of suitable capacity to obtain the portions necessary for the test.

Sterilize for 15 min in the autoclave (6.1) set at 121 °C.

Store the medium in closed flasks at 5 °C ± 3 °C (6.5) for up to 6 months.

B.1.4 Performance testing

For the definition of productivity, refer to ISO 11133. The performance of buffered peptone water (BPW) shall be tested in accordance with the methods described in ISO 11133. Table B.1 shows the performance criteria of buffered peptone water (BPW) used as enrichment medium for *Enterobacteriaceae*.

Table B.1 — Performance testing of buffered peptone water (BPW)

Medium	Function	Incubation	Control strains	WDCM[a] number	Method of control	Criteria[e]
Buffered peptone water (liquid medium)	Productivity	(18 ± 2) h / (37 ± 1) °C	*Escherichia coli* *Salmonella* Typhimurium[c,d] *Salmonella* Enteritidis[c,d]	00012[b] 00013 00031 00030	Qualitative	Turbidity 1 to 2

[a] Refer to the reference strain catalogue at :www.wfcc.info for information on culture collection strain numbers and contact details; WDCM: World Data Centre for Microorganisms.

[b] Strain to be used as a minimum.

[c] 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.

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

[e] Growth/turbidity is categorized as 0: no growth/no turbidity; 1: weak growth/slight turbidity; 2: growth/good turbidity.

B.2 Violet red bile glucose (VRBG) agar

B.2.1 Components

Enzymatic digest of animal tissues	7,0 g
Yeast extract	3,0 g
Bile salts	1,5 g
Glucose	10,0 g
Sodium chloride	5,0 g
Neutral red	0,03 g
Crystal violet	0,002 g
Agar	9 g to 18 g [a]
Water	1 000 ml

[a] Depending on the gel strength of the agar.

B.2.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by boiling.

Adjust the pH, if necessary, so that after boiling it is 7,4 ± 0,2 at 25 °C.

Dispense the culture medium into sterile tubes or flasks (6.5) of appropriate capacity.

Do not sterilize the medium.

Use the molten medium within 4 h of its preparation.

B.2.3 Preparation of agar plates

Immediately transfer portions of the culture medium, melted and cooled to 47 °C to 50 °C, to Petri dishes (6.7) 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), and allow to solidify.

Just before use, dry the plates, preferably with the lids off and the agar surface downwards, in a drying cabinet (6.3) until the agar is dry.

If prepared in advance, the undried plates may be stored in conditions that do not change their composition for up to 2 weeks at 5 °C ± 3 °C.

B.2.4 Performance testing for the quality assurance of the culture medium

For the definition of selectivity and productivity, refer to ISO 11133. Table B.2 shows the performance criteria of violet red bile glucose (VRBG) agar.

Table B.2 — Performance criteria of violet red bile glucose (VRBG) agar

Medium	Function	Incubation	Control strains	WDCM[a] number	Reference media	Method of control	Criteria[e]	Characteristic reactions
VRBG (solid medium)	Productivity	(24 ± 2) h / (37 ± 1) °C	*Escherichia coli* *Salmonella* Typhimurium[c,d] *Salmonella* Enteritidis[c,d]	00012[b] 00013 00031 00030	Tryptic Soy Agar (TSA)	Qualitative	Good growth (2)	Pink to red colonies with or without precipitation halo
	Selectivity		*Enterococcus faecalis*[d]	00009 or 00087		Qualitative	Total inhibition (0)	

[a] Refer to the reference strain catalogue at www.wfcc.info for information on culture collection strain numbers and contact details; WDCM: World Data Centre for Microorganisms.

[b] Strain to be used by the user laboratory (minimum).

[c] 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.

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

[e] Growth/turbidity is categorized as 0: no growth/no turbidity; 1: weak growth/slight turbidity; 2: growth/good turbidity.

B.3 Non-selective agar medium

B.3.1 General

The choice of the non-selective agar medium for purity check is left to the discretion of the testing laboratory. The manufacturer's instructions should be followed regarding its preparation for use. An example of a non-selective agar medium is nutrient agar (NA).

B.3.2 Composition nutrient agar

Meat extract	3,0 g
Enzymatic digest of animal tissues	5,0 g
Sodium chloride	5,0 g

Agar	9 g to 18 g [a]
Water	1 000 ml

[a] Depending on the gel strength of the agar.

B.3.3 Preparation

Dissolve the components or the dehydrated complete medium in the water by heating, with frequent agitation.

Adjust the pH, if necessary, so that after sterilization it is 7,0 ± 0,2 at 25 °C.

Transfer the culture medium into sterile tubes or flasks (6.5) of appropriate capacity.

Sterilize for 15 min in the autoclave (6.1) set at 121 °C.

B.3.4 Preparation of agar plates

Cool the medium to 47 °C to 50 °C in a water bath (6.4), mix and pour into sterile Petri dishes (6.7). Allow to solidify.

Immediately before use, dry the agar plates carefully (preferably with the lids off and the agar surface downwards) in the oven (6.3) set between 25 °C and 50 °C until the surface of the agar is dry.

Store the poured plates, protected for drying, at 5 °C ± 3 °C for up to 4 weeks.

B.3.5 Performance testing for the quality assurance of the culture medium

For the definition of productivity, refer to ISO 11133. Table B.3 shows the performance criteria of nutrient agar.

Table B.3 — Performance criteria of nutrient agar

Medium	Function	Incubation	Control strains	WDCM[a] number	Method of control	Criteria [c]
Nutrient agar (Solid medium)	Productivity	(24 ± 2) h / (37 ± 1) °C	*Escherichia coli*	00012[b] 00013	Qualitative	Good growth (2)

[a] Refer to the reference strain catalogue at www.wfcc.info for information on culture collection strain numbers and contact details; WDCM: World Data Centre for Microorganisms.

[b] Strain to be used by the user laboratory (minimum).

[c] Growth/turbidity is categorized as 0: no growth/no turbidity; 1: weak growth/slight turbidity; 2 growth/good turbidity.

B.4 Glucose OF medium

B.4.1 Composition

Enzymatic digest of casein	2,0 g
Dipotassium hydrogen phosphate (K_2HPO_4)	0,3 g
Glucose	10,0 g
Sodium chloride	5,0 g