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<th>Related content</th>
<th>Document date</th>
<th>Expected action</th>
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<tbody>
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<td>General / Other</td>
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<td>2022-08-26</td>
<td>INFO</td>
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</tbody>
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Description

Please find the 3rd edition of the ‘Guidance Document’: *Microbiology of the food chain — Template and guidance for drafting ISO/CEN standards* prepared by Ad'hoc Group "Guidance document...".

It has been already shared with the ISO editor.

It will be shared with WG convenors and liaisons according to resolution N2022/06.
Microbiology of the food chain — Template and guidance for drafting ISO/CEN standards

As the ‘CEN/ISO Guidance Document’ will be used by the Project Leaders and Secretariats within ISO/TC 34/SC 9 ‘Food products — Microbiology’ and CEN/TC 463 ‘Microbiology of the food chain’, it is necessary to bring it in line with the ISO/IEC Directives, SC 9-resolutions and CEN/TC 463-decisions at all times (yearly). In addition, the ‘CEN/ISO Guidance Document’ is made available as an N-document to all members of ISO/TC 34/SC 9 and CEN/TC 463.
## Contents

Background information and basics ........................................................................................................... 5
Title .................................................................................................................................................................. 9
Copyright notice ........................................................................................................................................... 9
Table of Contents ........................................................................................................................................ 9
Foreword ....................................................................................................................................................... 9
Introduction .................................................................................................................................................. 10
Warnings and safety precautions .................................................................................................................. 10
1 Scope ....................................................................................................................................................... 11
2 Normative references ............................................................................................................................... 13
3 Terms and definitions .................................................................................................................................. 14
4 Principle .................................................................................................................................................... 16
5 Culture media and reagents ....................................................................................................................... 16
6 Equipment and consumables ...................................................................................................................... 17
7 Sampling ................................................................................................................................................... 17
8 Preparation of test sample .......................................................................................................................... 18
9 Procedure .................................................................................................................................................. 18
10 Expression of results ................................................................................................................................. 19
11 Validation of the method ........................................................................................................................... 20
12 Test report ................................................................................................................................................. 22
13 Quality assurance ..................................................................................................................................... 23
Annexes ....................................................................................................................................................... 23
Annex A (normative) Flow diagram(s) of the procedure(s) ........................................................................... 23
Annex B (normative) Culture media and reagents ....................................................................................... 23
Annex C (informative) Performance characteristics of the method .............................................................. 27
Bibliography ................................................................................................................................................ 29

EXAMPLE: Annex 1 (informative) Examples for the content of clauses of an International Standard for a qualitative microbiological culture method .................................................................................. 30

## Foreword

Introduction ....................................................................................................................................................... 30
1 Scope ....................................................................................................................................................... 30
2 Normative references ............................................................................................................................... 31
3 Terms and definitions .................................................................................................................................. 31
4 Principle .................................................................................................................................................... 32
5 Culture media and reagents ....................................................................................................................... 32
6 Equipment and consumables ...................................................................................................................... 32
7 Sampling ................................................................................................................................................... 33
8 Preparation of test sample .......................................................................................................................... 33
9 Procedure .................................................................................................................................................. 33
10 Expression of results ................................................................................................................................. 35
11 Validation of the method ........................................................................................................................... 36
12 Test report ................................................................................................................................................. 36
13 Quality assurance ..................................................................................................................................... 36
Annex A (normative) Flow diagram of the procedure ................................................................................... 37
Annex B (normative) Culture media and reagents ....................................................................................... 38
Annex C (informative) Performance characteristics of the method .............................................................. 42
EXAMPLE: Annex 2 (informative) Examples for the content of clauses of an International Standard for a quantitative microbiological culture method

1 Scope..............................................................................................................................................43
2 Normative references.........................................................................................................................43
3 Terms, definitions and abbreviated terms..........................................................................................43
4 Principle...............................................................................................................................................44
5 Culture media and reagents................................................................................................................44
6 Equipment and consumables..............................................................................................................44
7 Sampling.............................................................................................................................................45
8 Preparation of test sample .................................................................................................................45
9 Procedure ...........................................................................................................................................45
10 Expression of results..........................................................................................................................46
11 Validation of the method ..................................................................................................................47
12 Test report ........................................................................................................................................47
Annex A (normative) Flow diagram of the procedure ...........................................................................48
Annex B (normative) Culture media and reagents.................................................................................48
Annex C (informative) Performance characteristics of the method......................................................50
Annex 3 (informative) Microbiological terms and abbreviated terms.................................................51
Bibliography..........................................................................................................................................52
**Background information and basics**

This document is a guidance document for drafting International Standards under ISO/TC 34/SC 9, *Food products — Microbiology*, and CEN/TC 463, *Microbiology of the food chain*. The Guidance Document is intended to (further) harmonize the content and layout of International Standards for microbiology of the food chain. It generates, for example, the basic fixed text to be used, see: [Simple template](http://www.iso.org/iso/model_document-rice_model.pdf) [Word]. The simple template is also applicable to other ISO deliverables, e.g. ISO/Technical Specification or ISO/Technical Report.

To refer to information in an ISO document, 'this document' shall be used as a generic term to refer to 'this International Standard', 'this Technical Report', etc.

This Guidance Document has been drafted in particular for culture methods for detection and enumeration of microorganisms. The ‘template’ can be adapted for specific situations. This Guidance Document specifies the elements that are used in ISO documents, International Standards, ISO/Technical Specifications, etc. The focus of this Guidance Document is on how to write International Standards for microbiology of the food chain.

The following documents were consulted for drafting this Guidance Document:

- ISO 78-2, *Chemistry — Layouts for standards — Part 2: Methods of chemical analysis*;

Existing (draft) ISO documents for microbiology of the food chain and agreements (mostly published as Resolutions or Decisions) made during meetings of ISO/TC 34/SC 9 and CEN/TC 463 have also been considered in the development of this Guidance Document. After drafting an ISO document, a final (editorial) check will be performed by the Secretariat of the Working Group, by the Committee Manager of ISO/TC 34/SC 9, and by the editor of ISO Central Secretariat. During this check, the following actions will be done: the standard text of the Foreword and the Table of Contents will be updated, and the layout of references and the Bibliography will be finalized.

In this Guidance Document, the different clauses of an ISO document for microbiology of the food chain are described, and guidance is given on the content of each clause. In some clauses, ‘standard sentences’ are included, agreed by either ISO Central Secretariat, CEN Management Centre or by the members of ISO/TC 34/SC 9 and CEN/TC 463. For the sake of clarity, these ‘standard sentences’ are given in *italics*, although they shall not be italicized in an official ISO document.

Examples of contents of clauses and subclauses are given in Annexes 1 and 2. The start of each example throughout this Guidance Document is indicated with *Example* or *for example*. Annex 3 provides a list of microbiological terms and abbreviated terms that are used in ISO documents for microbiology of the food chain. This information is summarized to make sure that specific terms and abbreviated terms are described/used in the same way in different ISO documents for microbiology of the food chain. It is a ‘dynamic’ annex, where content can change over time.
The *English language* used in ISO documents is ‘Oxford English’, which is similar to standard British English except words such as ‘organization’ and ‘standardization’ follow the Greek root and are written with a ‘z’ (see the following [link](#) for further information).

The main text of ISO documents is structured in numbered *clauses and subclauses*, which can contain normative and informative parts. A normative text contains requirements and an informative text gives guidance. Throughout the document, it shall be clear what is a requirement, a recommendation or another type of statement. ISO uses the following provisions (verbal forms) to make the distinction:

— requirements (shall, shall not);
— recommendations (should, should not);
— permissions (may);
— possibility and capability (can, cannot).

To express an instruction, e.g. referring to steps to be taken in a test method, use the imperative mood in English. The imperative mood is frequently used to express requirements in test methods.

Do not use ‘must’ as an alternative for ‘shall’. This will avoid any confusion between the requirements of an ISO document and external constraints. Do not use ‘may not’ instead of ‘shall not’ to express a prohibition. Negative permissions are ambiguous and should not be used. Rather than using negative permissions, either rewrite the sentence to state what is permitted, or rewrite as a requirement or recommendation not to do something.

When specific information or a procedure is described in another ISO document and cited normatively in the document, the reference shall be cited normatively in the text using “shall” or a verb in the imperative tense, e.g. ‘ISO 7218 shall be used’ or ‘follow the procedures in accordance with ISO 7218’. Note that ‘see’ (e.g. ‘see ISO 7218’) or ‘refer to’ is considered as informative.

References to normative ISO documents are cited in Clause 2 ‘Normative references’ and to informative ISO documents in the Bibliography. Citation in the text to informative ISO documents do not need a reference number between brackets as the ISO standard itself already has a number which is sufficient.

When in an ISO document reference is made to a clause, the word ‘Clause’ is added before the number (e.g. Clause 4). For subclauses (e.g. 5.3 or B.2.2), only the number is given in a reference. When a reference is made to an annex as a whole, the word ‘Annex’ is used before the letter (e.g. Annex A). When reference is made to a clause in an annex, the word ‘Clause’ is added before the number (e.g. Clause B.2).

Use, for example, the following forms for references to clauses and subclauses in an ISO document:

— “in accordance with Clause 4”;
— “the methods described in 5.3 and 9.2.2.2 provide further information on”;
— “details as given in 4.1.1 and B.2.2”;
— “the flow diagram is given in Annex A”;
— “the requirements given in Clause B.2”.

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Guidance Document, 3rd edition
Notes and Examples are used for giving additional information intended to assist the understanding or use of the document and shall not contain requirements, instructions (imperative), recommendations or permissions. It is also allowed to refer to a specific document rather than repeating large portions of text. Notes and Examples are given in a font one size smaller than the main text. If more than one Note is given in a (sub)clause, they shall be numbered consecutively. For example:

NOTE 1 Some strains show....

NOTE 2 Some organisms are ....

Notes to terms and definitions are called ‘Notes to entry’ and follow different rules from Notes integrated in the main text. They provide additional information that supplements the terminological data. A Note to entry may provide, for example, provisions relating to the use of a term, information regarding the units applicable to a quantity or an explanation of the reasons for selecting an abbreviated form as preferred term. In contrast with a Note in the main text, a Note to entry may contain requirements or recommendations.

Notes to entry are designated 'Note # to entry:' and shall be numbered starting at ‘1’ within each terminological entry. A single Note to entry shall also be numbered. Notes to entry shall be placed on a new line, after any Examples.

Unnumbered subdivisions of a clause or subclause ('Hanging paragraphs') shall be avoided, since reference to them is ambiguous. An example of a hanging paragraph:

4 Principle
The detection of Salmonella requires four successive steps as specified in Annex A.

4.1 Pre-enrichment

The hanging paragraph ('The detection... ...Annex A.') cannot be uniquely identified. To avoid this problem, it is necessary to identify the hanging paragraph as subclause ‘4.1 General’ (or other suitable title) under Clause ‘4 Principle’ and to renumber the following subclauses accordingly.

Symbols and abbreviated terms used in an ISO document can be listed in alphabetical order in a separate subclause. An introductory sentence is not needed. If a list of abbreviated terms is not given in the ISO document, then the first time that an abbreviated term is used, the full term shall be given with the abbreviated term following in brackets, e.g. colony-forming units (cfu).

Tables and figures shall have a number and a title. For a table, the title shall be given above the table. For a figure, the title shall be given below the figure. Examples are given in Annex 1 and Annex 2. Tables and figures shall be cited in the text. If footnotes are used in a table, they are indicated by letters and not by numbers.

For decimal values, a decimal comma is used throughout ISO documents, instead of a decimal point. To facilitate the reading of numbers with many digits, these are separated into groups of three, separated by a small space and not by a point or a comma or by any other means (e.g. 1 000).

Use ‘CTRL-SHIFT-SPACE’ instead of a regular space to make sure that items stay together on a line; this is called a non-breaking space. For example, values and units (e.g. 10 ml, 18 h), tolerances (e.g. 18 h ± 2 h), names of bacteria (e.g. S. Typhimurium).
For the nomenclature of microorganisms, follow the international rules as published:

— for *Salmonella*: by the WHO Collaborating Centre for Reference and Research on *Salmonella* (White Kauffmann-Le Minor scheme), see [https://www.pasteur.fr/sites/default/files/veng_0.pdf](https://www.pasteur.fr/sites/default/files/veng_0.pdf). This is also summarized in ISO/TR 6579-3 (Serotyping of *Salmonella*).

— for all other Prokaryotes: by the International Committee on Systematics of Prokaryotes (ISCP), see [https://www.the-icsp.org/](https://www.the-icsp.org/), and List of Prokaryotic names with standing in nomenclature, see [https://lpsn.dsmz.de/text/nomenclature](https://lpsn.dsmz.de/text/nomenclature).

The List of Prokaryotic names with Standing in Nomenclature also provides information on the following abbreviations:

— sp.: species;
— spp.: species plural;
— subsp.: subspecies;
— subspp.: subspecies plural.

For ISO documents for microbiology of the food chain, especially ‘sp.’ and ‘spp.’ are of relevance and are used as follows:

— ‘sp.’ is used when the actual specific name cannot or does not need to be specified, so it is all the species of the genus. Here it is better to write only the genus name (without ‘sp.’);
— ‘spp.’ (plural) means ‘several species’ and is used when the ISO document may not be applicable to all the species of the genus.

These abbreviations are not italicized (or underlined). For example: ‘*Salmonella* sp.’ means ‘an unspecified species of the genus *Salmonella*, while ‘*Salmonella* spp.’ means ‘two or more species of the genus *Salmonella*’.

The use of services, such as testing and certification by another company, shall not be mandated in an ISO document. Therefore, give the requirements so they can be verified by anyone. Reference to trademarks, companies or patented items should be avoided. If it is absolutely necessary to include these elements in the ISO document, contact the ISO/TC 34/SC 9 Committee Manager or ISO Central Secretariat for further guidance.

A published ISO document may subsequently be modified by the publication of a corrected version or an amendment before a revision of the whole ISO document is carried out. A corrected version replaces the ISO document, while an amendment is a separate document that supplements the edition of the ISO document affected. A corrected version is issued to correct a technical error or ambiguity, inadvertently introduced either in drafting or in publishing of an ISO document, and which could lead to incorrect or unsafe application of the publication. An amendment alters and/or adds to previously agreed technical provisions in an existing ISO document. No more than two separate documents in the form of corrections or amendments shall be published modifying a current ISO document. The development of a third such document shall result in publication of a new edition of the ISO document.

More detailed information on the ISO House Style (regarding plain English, grammar, spelling, punctuation, referencing, formatting, etc.) is available online: [https://www.iso.org/ISO-house-style.html](https://www.iso.org/ISO-house-style.html).
Microbiology of the food chain — Template and guidance for drafting ISO/CEN standards

Title

The title shall be clear and concise and is limited to a maximum of three elements to reflect the Scope:

a) An introductory element, which is fixed by ISO/TC 34/SC 9 and CEN/TC 463 as ‘Microbiology of the food chain’.

b) A main element that describes the purpose of the document, for example: ‘Horizontal method for the detection and enumeration of [microorganism(s)]’

c) A complementary element that expands on the main element, for example: ‘Part 1: Detection method’.

If an ISO document consists of several parts, it is usual that Part 1 is the detection method and Part 2 the enumeration method, for example: ‘Part 2: Enumeration by colony-count technique’, ‘Part 3: Enumeration by most probable number technique’.

Copyright notice

The copyright notice is part of the ISO template. An abbreviated copyright notice shall also appear on each page, as footer, as follows: © ISO [year] – All rights reserved. This is also included in the basic fixed text of the template.

Table of Contents

The Table of Contents is generated automatically to three levels when processed at ISO Central Secretariat. Start the Table of Contents on a new page.

Foreword

Start the Foreword on a new page. A Foreword shall appear in each ISO document and shall not contain requirements, recommendations or permissions. It is therefore an informative element. The generic text is inserted by ISO Central Secretariat during editing and publishing. The specific text is supplied by the committee and includes:

— The name of ISO/TC 34/SC 9 when the work is performed only on the international level. If the work is performed under Vienna Agreement, both ISO/TC 34/SC 9 and CEN/TC 463 are mentioned.

— A statement that the document cancels and replaces other documents in whole or in part (including the edition number).

— The main changes compared with the previous edition, using the following ‘standard sentence’: “The main changes are as follows:”.

For a series, reference is made to the ISO website. The user can find an up-to-date list of parts of the ISO series there (see the following link for an example).
Introduction

Start the Introduction on a new page. The Introduction is optional, but ISO encourages its inclusion. It is an informative element and shall not contain any requirements. The Introduction can describe the content of the document and explain why the document is needed. The Introduction shall not contain any disclaimers or statements on the limitations of the method; these shall be given in the Scope. The Introduction is only mandatory if a specific patent right has been identified during the development of the document. Where patent rights have been identified in a document, the Introduction shall include an appropriate notice. In this case, contact the ISO/TC 34/SC 9 Committee Manager or ISO Central Secretariat for further guidance.

Information shall be given on the nature of the changes compared with the previous edition (see ISO 17468), and on their impact on the method performance. Editorial changes will be classified as minor changes. Technical changes will be classified as minor or major.

Use the following 'standard sentences' to describe the nature of the technical changes:

The main technical changes listed in the Foreword, introduced in this document compared with ISO ####:[year], are considered as [minor/major] (see ISO 17468).

These technical changes have a major impact on the performance characteristics of the method. OR
These technical changes have a minor impact on the performance characteristics of the method.

Warnings and safety precautions

The content of a warning shall describe the microorganism hazard, the risk presented by exposure to the hazard and the consequences if the warning is not followed. If the microorganism being analysed, or if the reagents or the procedure are dangerous, either to health or to the environment, it is essential to draw attention to the hazards and to describe the precautions necessary to avoid them. This information shall be printed in bold type and placed:

— immediately after the title of the ISO document (above the Scope) if the danger is of a general nature or is due to the product being analysed;
— after the name of the reagent or material if the danger is due to a particular reagent or material;
— at the beginning of the 'Procedure' clause if the danger is inherent in the procedure.

In ISO documents for microbiology of the food chain, the following warning describing the danger of a general nature is often given immediately after the title of the ISO document:

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for [detection/enumeration/typing] of [microorganism] 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.

If necessary, more detailed advice on safety procedures and first-aid measures may be given in an annex.
1 Scope

The Scope is a mandatory element which describes what the document does (‘specifies’, ‘establishes’, ‘gives guidelines for’, ‘defines terms’). It shall be worded as a series of statements of fact and not contain requirements, recommendations or permissions. The limits of applicability of the ISO document or its particular parts shall be indicated in the Scope. It shall contain sufficient information to enable the user to judge quickly whether the ISO document is applicable to the products being considered or whether limitations exist. If necessary, the Scope should indicate subjects that might be reasonably inferred to be covered but actually excluded from the ISO document (‘excludes’, ‘does not apply to’).

If an ISO document for microbiology of the food chain is applicable to all different areas of the food chain, the following ‘standard sentences’ shall be used:

This document is applicable to:

— products intended for human consumption;
— products for feeding animals;
— environmental samples in the area of food and feed production and handling;
— samples from the primary production stage.

If an ISO document for microbiology of the food chain is not applicable to all different areas of the food chain, indicate only the areas for which the document is applicable. For example:

This document is applicable to:

— products intended for human consumption;
— products for feeding animals.

If an ISO document for microbiology of the food chain is only applicable to one (food) category, indicate only the category for which the document is applicable. For example:

This document is applicable to fresh produce and fruits.

In this situation, it should also be considered if a change in the title of the ISO document is required to emphasize the limitations of the applicability of the method. For example: ISO 19343:2017, Microbiology of the food chain — Detection and quantification of histamine in fish and fishery products — HPLC method.

If the ISO method is validated for ‘a broad range of food’, that is, the validation studies included at least five different food categories, and if the ISO method is validated for other categories as well, the following ‘standard NOTE’ shall be added:

NOTE This method has been validated in an interlaboratory study for the following food categories:

— [category 1];
— [category 2];
— [category 3];
Guidance Document, 3rd edition

— [category 4];
— [category 5].

It has also been validated for the following other categories:
— [pet food and animal feed];
— [environmental samples (food or feed production)];
— [primary production samples (PPS)].

As this method has been validated for at least five food categories, this method is applicable for a broad range of food. For detailed information on the validation, see Clause 11 and Annex C.

If the ISO method is validated for a limited number of (food) categories, the following ‘standard NOTE’ shall be added:

NOTE   This method has been validated in an interlaboratory study for the following food categories:
— [category 1];
— [category 2];
— [category 3];
— [category 4].

It has also been validated for the following other categories:
— [pet food and animal feed];
— [environmental samples (food or feed production)];
— [primary production samples (PPS)].

For detailed information on the validation, see Clause 11 and Annex C.

When limitations of the applicability of the method are known, the following ‘standard sentences’ shall be used:

This horizontal method was originally developed for the examination of all samples belonging to the food chain. However, because of the large variety of products in the food chain, it is possible that this horizontal method is not appropriate in every detail for all products. Nevertheless, it is expected that the required modifications are minimized so that they do not result in a significant deviation from this horizontal method.

Based on the information available at the time of publication of this document, this method is not considered to be (fully) suited to the examination of ... [e.g. ‘Raw and ready-to-cook fish and seafoods (unprocessed)’ or ‘Fish (unprocessed)’ or ‘Raw tuna’]. Any limitations are further explained in Annex # of this document.

Please note that the wording for the limitation of the method should reflect the specificity of the limitations. For example, whether this is due to a particular (food) matrix/item, e.g. ‘raw tuna’, or a (food) type within a category, e.g. ‘fish (unprocessed)’ or even a whole (food) category, e.g. ‘raw and ready-to-cook fish and seafoods (unprocessed)’. If it concerns a particular (food) item, the (food) item or (food)
items should be mentioned and not the type and category it belongs to (see the ‘standard sentences’ mentioned above).

When no limitations of the applicability of the method are known, the following ‘standard sentences’ shall be used:

*This horizontal method was originally developed for the examination of all samples belonging to the food chain. Based on the information available at the time of publication of this document, this method is considered to be fully suited to the examination of all samples belonging to the food chain. However, because of the large variety of products in the food chain, it is possible that this horizontal method is not appropriate in every detail for all products. Nevertheless, it is expected that the required modifications are minimized so that they do not result in a significant deviation from this horizontal method.*

For information on limitations or suitability of techniques, the following ‘standard sentences’ shall be used (e.g. for several parts in a series of ISO documents):

**For most probable number (MPN) technique:**

*This technique is suitable for, but not limited to, use when the result is expected to be below 10 microorganisms per millilitre for liquid samples or below 100 microorganisms per gram for solid samples.*

**For colony-count technique (pour plates):**

*This technique is suitable for, but not limited to, the enumeration of microorganisms in test samples with a minimum of 10 colonies counted on a plate. This corresponds to a level of contamination that is expected to be higher than 10 cfu/ml for liquid samples or higher than 100 cfu/g for solid samples.*

**For colony-count technique (spread plates):**

*This technique is suitable for, but not limited to, the enumeration of microorganisms in test samples with a minimum of 10 colonies counted on a plate. This corresponds to a level of contamination that is expected to be higher than 100 cfu/ml for liquid samples or higher than 1 000 cfu/g for solid samples.*

2 Normative references

This clause is mandatory and lists the documents that are required for the application of the ISO document and are cited normatively in the document. References should only be to other ISO documents. However, in case of an exception, contact the ISO/TC 34/SC 9 Committee Manager. Remember to date the references if reference is made to a specific element, e.g. clause or subclause, figure, table or annex. Documents which have merely served as references in the preparation of the ISO document shall be indicated in the Bibliography at the end of the ISO document (for example, referenced documents that are only cited in an informative manner or those that provide background information on the topic).

References shall be publicly available; this means for ISO documents that they have at least reached the enquiry stage (e.g. ISO/DIS). Before listing the referenced ISO documents, the following ‘standard sentences’ shall be given:
Guidance Document, 3rd edition

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.

The above wording is also applicable to a part of a multipart document (ISO series).

If no references exist, include the following phrase below the clause title:

There are no normative references in this document.

Where reference is made to all parts of an ISO document with multiple parts (series), the normative reference shall be written as follows: ISO ###### (all parts). For example:

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

3 Terms and definitions

The clause on terms and definitions is a mandatory element, even if the ISO document contains no terminological entries. This clause gives definition(s) necessary for the understanding of certain terms used in the document. If possible, give preference to existing definitions and/or terms in other ISO documents (you can search in www.iso.org/obp to determine if a term has already been defined).

A definition is a single phrase that can replace the term wherever used and shall not contain a requirement or recommendation. Additional information shall only be given in the form of examples or notes to entry (see ‘Background information and basics’). Each term shall be numbered, written in bold and given in the singular form (except for terms that are only used in the plural form, e.g. ‘goods and services’). If necessary, an abbreviation of a term can also be given, written in bold, below the term. Terms and definitions shall be written without a capital letter (except for terms that are proper nouns, e.g. ‘Reynolds number’) and without a period (full stop) at the end of the definition. A definition shall not start with an article (‘the’, ‘a’). The use of alphabetical order of terms and definitions is discouraged because the order has to remain the same for translated versions, in which alphabetical order in English is meaningless. The terms and definitions are rather listed by ‘the hierarchy of the concepts (i.e. systematic order)’ – i.e. the most important items first, grouped by subject.

Until 2018, definitions of microorganisms in ISO documents for microbiology of the food chain generally included a phrase such as: ‘only tests carried out following this document’. This is no longer allowed, because:

— Requirements are not allowed in definitions (e.g. ‘in accordance with’). These shall be specified in the relevant (sub)clause of the document.

— Definitions should be drafted in a single phrase so that they can replace the word being defined in the text.

— When published on the Online Browsing Platform (www.iso.org/obp), definitions are standalone definitions with no reference possible to the content of the document they come from, e.g. ‘following this document’ – which document?

For the ‘new’ definitions of microorganisms the following has been agreed:

— general information of the microorganism is given in the definition and in one sentence;
— requirements (such as a reference to a relevant clause of the ISO document) are given in notes to entry;

— in the definition, the main characteristics of the microorganism related to the method of that ISO document are described;

— characteristics which are not tested in accordance with the method of that ISO document are not part of the definition. For example, information on Gram positivity or Gram negativity is not given in the definition when Gram staining is not part of the described method.

Different cases can be found:

— An existing term or definition from another ISO document (see example in 3.1). In this example it is also shown if any changes are made to the original terminological entry, this shall be indicated, along with a description of what has been modified.

— A ‘new’ definition for a microorganism (see example in 3.2).

— For toxins, where the specific component is mentioned as well as the specific organism or group of organisms producing the toxin (see example in 3.3).

Add the following ‘standard sentences’ before listing the terms and definitions:

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

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

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

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

3.1 analyte
component represented in the name of a measurable quantity

[SOURCE: ISO 17511:2020, 3.1, modified — The example has been deleted.]

3.2 Salmonella
genus of microorganisms of the family Enterobacteriaceae, usually capable of growth in/on Rappaport-Vassiliadis medium with soya (RVS) broth, modified semi-solid Rappaport-Vassiliadis (MSRV) agar and Muller-Kauffmann tetrathionate-novobiocin (MKTn) broth, forming typical or less typical colonies on solid selective media, such as xylose lysine deoxycholate (XLD) agar, and displaying certain characteristics with biochemical and serological confirmation tests

Note 1 to entry: The biochemical and serological confirmation tests and the characteristics of Salmonella are described in 9.5.

3.3 cereulide
toxin cyclo[O-Leucine-D-Alanine-L-O-Valine-L-Valine], produced by certain strains of the species of Bacillus cereus

[SOURCE: ISO 18465:2017, 3.1]
4 Principle

This clause indicates the essential steps in the method used, the basic principles and the properties relied upon and, if appropriate, the reasons justifying the choice of certain steps in the procedure. For the sake of clarity, give the different steps of the procedure in different subclauses.

In this clause, no tolerances for incubation temperatures or for incubation times are given, as only the general principles of the method are described.

If an ISO document consists of multiple parts (series), which contain two or three technique options (spread plate, pour plate and/or MPN), the quantification limits for the technique are given in the relevant part, in Clause 4, using the following ‘standard sentences’:

**For colony-count technique (spread plates):**

*If a lower limit of 10 cfu/ml (liquid samples) or 100 cfu/g (solid samples) is required, 1 ml can be examined using one large Petri dish (140 mm diameter) or three 90 mm diameter Petri dishes.*

*When the number of cfu is expected to be at or near the limit of determination, the use of duplicate plates is preferable. If duplicate plates are used, the minimum count for the sum of colonies on both plates should be 10 colonies. In this case, the level of contamination is expected to be higher than 50 cfu/ml for liquid samples or higher than 500 cfu/g for solid samples.*

*This technique is especially suited for the enumeration of heat-sensitive microorganisms.*

**For colony-count technique (pour plates):**

*When the number of cfu is expected to be at or near the limit of determination, the use of duplicate plates is preferable. If duplicate plates are used, the minimum for the sum of colonies on both plates should be 10 colonies. In this case, the level of contamination is expected to be higher than 5 cfu/ml for liquid samples or higher than 50 cfu/g for solid samples.*

*A pour-plate technique is especially suited for the enumeration of products expected to contain spreading colonies that can obscure colonies of the target microorganisms.*

5 Culture media and reagents

In this clause, only general information is given, as the composition and preparation of culture media and reagents shall be specified in a normative annex. Whenever possible, avoid the use of toxic chemicals for preparation of culture media and reagents.
Abbreviations of culture media are given in capitals, for example: XLD. The full name of culture media should be in lower case letters (the first letter will be in capitals when it is at the start of a sentence or title of a subclause). For example: xylose lysine deoxycholate. When the full name of a medium contains a name of a person or microorganism, this word/these words should be in capitals. For example: Rappaport-Vassiliadis soya peptone broth (RVS).

In ISO documents for microbiology of the food chain, the following ‘standard sentences’ will, generally, be sufficient for this clause:

Follow current laboratory practices in accordance with ISO 7218. The composition of culture media and reagents and their preparation are specified in Annex #. For performance testing of culture media, follow the procedures in accordance with ISO 11133 and Annex #.

6 Equipment and consumables

NOTE 1 Before 2015, the title of this clause was ‘Apparatus and glassware’.

This clause shall list the names and significant characteristics of all the equipment and consumables, other than usual microbiological laboratory equipment, to be used during the analysis. The items of equipment and consumables shall be identified by a (sequential) reference number. The items can be alphabetically ordered, but this is not a rule. Repetition of the characteristics of the equipment and/or consumables in question is avoided by inserting this number, in parentheses, in the ‘Procedure’ clause after the name of the item.

Special requirements for any equipment/consumable that is critical to the method shall be given in this clause, especially if they have a significant role in the procedure. Requirements for temperature tolerances of incubators, refrigerators and freezers are given in this clause and not in the ‘Procedure’ clause as these requirements are part of the apparatus specifications. This clause shall start with the following ‘standard sentences’ before listing the equipment and consumables:

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

NOTE 2 This introductory text is not a hanging paragraph, since it is introducing a list detailing the equipment and consumables and is not a series of subclauses.

If instead of temperature tolerances, a temperature range is given for an incubator, a note is added to clarify which temperature tolerances are included. For example:

6.1 Incubator, capable of operating in the range 34 °C to 38 °C.

NOTE The range 34 °C to 38 °C for incubation of culture media includes the use of incubators set at 35 °C ± 1 °C, 36 °C ± 2 °C or 37 °C ± 1 °C.

7 Sampling

Use general wording and, if necessary, provide additional information that is specific for the microorganism(s) of concern. When possible, refer to ISO documents for sampling.

In ISO documents for microbiology of the food chain, sampling shall be expressed in general with the following ‘standard sentences’:
Sampling is not part of the method specified in this document. Follow 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 the following documents:

— ISO/TS 17728 for food and animal feed;
— ISO 707 for milk and milk products;
— ISO 6887-3 for raw molluscs, tunicates and echinoderms from primary production areas;
— ISO 13307 for primary production stage;
— ISO 17604 for carcasses;
— ISO 18593 for surfaces.

It is important that the laboratory receives a sample that is representative of the product under consideration. The sample should not have been damaged or changed during transport or storage.

8 Preparation of test sample

Start this clause with the following ‘standard sentences’:

Prepare the test sample from the laboratory sample in accordance with the specific International Standard dealing with the product concerned. Follow the procedures specified in the ISO 6887 series. If there is no specific International Standard available, it is recommended that the parties concerned come to an agreement on this subject.

9 Procedure

This clause may be divided into as many subclauses as there are operations or sequences of operations to be carried out. Each sequence of operations shall be described unambiguously and concisely, using the imperative mood. If there are numerous steps in the procedure, it is recommended that the subclause covering each step be further subdivided, using the point numbering system, with each point corresponding to a given operation. Include all required preliminary operations. The numbered subclauses will facilitate cross-referencing later in the text.

If the method to be described is already given in another ISO document, the phrase ‘Use the method specified in ISO #######’ shall be used, with any necessary modifications.

If there are hazards or risks during the procedure for which special precautions are necessary, a cautionary statement shall be included in bold type at the beginning of the clause.

Temperature tolerances of incubators, refrigerators or freezers (e.g. ±1 °C) shall not be given in this clause or subclauses but give the ‘set’ temperature (e.g. 37 °C) and refer to the relevant numbered equipment or consumable under Clause 6, e.g. (6.1). If no ‘set’ temperature needs to be given, indicate the minimum and maximum temperatures between which the materials need to be incubated or stored, for example: ‘Incubate between 34 °C and 38 °C.’
Where relevant, give tolerances for the incubation times, unless there is a technical reason not to do so. For example: $18 \, \text{h} \pm 2 \, \text{h}; 24 \, \text{h} \pm 2 \, \text{h}, 72 \, \text{h} \pm 3 \, \text{h}$.

The ‘default’ incubation conditions are aerobic in ISO documents for microbiology of the food chain; only conditions that are different from the default conditions shall be indicated in the specific ISO document.

Often the first subclause of the ‘Procedure’ clause includes information on test portion, initial suspension and dilutions. Whenever possible, refer to the different parts of the ISO 6887 series (Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination). If preparation of the test portion is specific for the microorganism or the procedure and if this is not (yet) described in the ISO 6887 series, give the details of preparation in this (sub)clause.

For qualitative methods, the ‘sample-to-enrichment’ ratio should be indicated as follows:

In general, an amount of test portion (mass or volume) is added to a quantity of [diluent] (mass or volume) to yield a ten-fold dilution. For example: a 25 g test portion is mixed with 225 ml of [diluent].

For a qualitative detection (by culture) method, the following ‘standard sentences’ (including the note) in relation to the size of the test portion should be given:

This document has been validated for test portions of 25 g or ml [or another mass or volume]. A smaller test portion may be used, without the need for additional validation/verification, providing that the same ratio between (pre-)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 [name of the organism].

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

[Remark of the AHG-convenor and secretariat: The grey-highlighted text will be aligned with DAmd.1 to ISO 6887-1:2017 and DAmd.1 to ISO 16140-4:2020 when these are available in 2023.]

To indicate the possible use of alternative confirmation procedures, the following ‘standard sentences’ and/or note should be used (the information in the note can also be given as main text):

If shown to be reliable, miniaturized galleries for the biochemical identification of [name of the organism] may be used (see ISO 7218).

NOTE Alternative procedures (see ISO 7218) can be used to confirm the isolate as [microorganism], provided that the suitability of the alternative procedure has been validated (see ISO 16140-4 or ISO 16140-6).

10 Expression of results

For quantitative tests, the following ‘standard sentences’ for expression of results should be used:

For calculation of the results, follow the procedure(s) in accordance with ISO 7218. Calculate and report the results as the number of [microorganism] in cfu per gram, per millilitre or per square centimetre. When the sampled area is not known, report as per sampling device, such as a cloth, sponge swab or stick.

In cases where no colonies of the target organism have been detected, follow ISO 7218 for the expression of results for special cases.
Guidance Document, 3rd edition

For qualitative tests, the following 'standard sentence' for expression of the results should be used:

In accordance with the interpretation of the results, indicate [microorganism] detected or not detected in a test portion of x g or x ml of product, or on the surface area swabbed or per sampling device.

11 Validation of the method

The method should be validated following ISO 17468. In this clause, information on the studies performed before the interlaboratory study and a summary of the performance characteristics is given. The detailed performance characteristics as well as possible background information are given in an annex.

When it is necessary to clarify that 'food categories' do not only concern food, but can also concern animal feed or samples from the primary production stage, the following note should be included in this clause (for qualitative methods as well as for quantitative methods):

NOTE In this document, the words "category", "type" and/or "item" are sometimes combined with "(food)" to improve readability. However, the word "food" is interchangeable with "feed" and other areas of the food chain as mentioned in Clause 1.

The following subclauses and 'standard sentences' should be applied for a qualitative culture method validated in accordance with ISO 17468.

11.1 Validation in accordance with ISO 17468

This standardized reference method was validated in accordance with ISO 17468. All relevant data as obtained in steps 1 to 5 of ISO 17468 were reported in [a scientific publication or in a report to be made available on the ISO Maintenance Portal associated to each standardized method].

The results of the interlaboratory study (step 6 in ISO 17468) have been published in [to be completed]. The performance characteristics of the method as derived from the interlaboratory study are described in 11.2.

11.2 Performance characteristics

The performance characteristics of the method (specificity, sensitivity and LOD$_{50}$) were determined in an interlaboratory study (or studies). All data are given in Annex #. It is possible that the values derived from the interlaboratory study are not applicable to (food) categories other than those used in the study (or studies).

A summary of the LOD$_{50}$ values is given in Table #.

<table>
<thead>
<tr>
<th>(Food) category</th>
<th>(Food) item</th>
<th>LOD$_{50}$ in cfu/test portion</th>
<th>Test portion size</th>
<th>Strain used in the interlaboratory study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>
The following subclauses and ‘standard sentences’ should be applied for a **quantitative culture method** validated in accordance with ISO 17468.

### 11.1 Validation in accordance with ISO 17468

This standardized reference method was validated in accordance with ISO 17468. All relevant data as obtained in steps 1 to 5 of ISO 17468 were reported in [a scientific publication or in a report to be made available on the ISO Maintenance Portal associated to each standardized method].

The results of the interlaboratory study (step 6 in ISO 17468) have been published in [to be completed]. The performance characteristics of the method as derived from the interlaboratory study are described in 11.2.

### 11.2 Performance characteristics

The performance characteristics of the method (repeatability and reproducibility standard deviations) were determined in an interlaboratory study (or studies). It is possible that the values derived from the interlaboratory study are not applicable to concentration ranges and (food) categories other than those used in the study (or studies). All data are given in Annex #.

A summary of the interlaboratory repeatability standard deviations ($s_r$) is given in Table #.

<table>
<thead>
<tr>
<th>(Food) category</th>
<th>(Food) item</th>
<th>$s_r$ values from the interlaboratory study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low inoculation level</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A summary of the interlaboratory reproducibility standard deviations ($s_R$) is given in Table #.

<table>
<thead>
<tr>
<th>(Food) category</th>
<th>(Food) item</th>
<th>$s_R$ values from the interlaboratory study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low inoculation level</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The following subclauses and ‘standard sentences’ should be applied for a **confirmation or typing method** validated in accordance with ISO 17468.
11.1 Validation in accordance with ISO 17468

This standardized reference method was validated in accordance with ISO 17468. All relevant data as obtained in steps 1 to 5 of ISO 17468 were reported in [a scientific publication, or in a report to be made available on the ISO Maintenance Portal associated to each standardized method or in a table added to the standardized reference method].

The results of the interlaboratory study (step 6 in ISO 17468) have been published in [to be completed]. The performance characteristics of the method as derived from the interlaboratory study are described in 11.2.

11.2 Performance characteristics

The performance characteristics of the method (inclusivity and exclusivity) were determined in an interlaboratory study (or studies). All data are given in Annex #.

A summary of the inclusivity and exclusivity data is given in Table #.

<table>
<thead>
<tr>
<th>Performance characteristic</th>
<th>Number of different strains</th>
<th>Total number of results</th>
<th>Inclusivity agreement</th>
<th>Inclusivity deviation</th>
<th>Exclusivity agreement</th>
<th>Exclusivity deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inclusivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Exclusivity</td>
<td></td>
<td></td>
<td>Not applicable</td>
<td>Not applicable</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

12 Test report

The following 'standard sentences' should be used as a model, with extra entries added on a case-by-case basis.

The test report shall specify at least the following:

— the test method used, with reference to this document, i.e. ISO ######;

— 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 or informative (including informative annexes), together with details of any incidents which can have influenced the test result(s);

— any deviations from this document;

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

— the test result(s) obtained;

— the date of the test;

— when necessary or if requested by the client, an estimate of the measurement uncertainty of quantitative test results, in accordance with ISO 19036:2019, Clause 9.
13 Quality assurance

The following 'standard sentences' shall be used for this clause:

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

Annexes

Start each annex on a new page. Annexes can be normative or informative elements. Normative annexes provide additional normative text to the main text of the ISO document. Informative annexes provide additional information intended to assist with the understanding or use of the ISO document. Informative annexes may contain optional requirements. For example, a test method that is optional may contain requirements but it is not necessary to follow these requirements to claim conformance with the ISO document.

The status of the annex (informative or normative) shall be made clear by the way in which it is referred to in the main text and shall be stated under the heading of the annex. All annexes shall be cited in the text. Annexes are designated by a capital letter (A, B, C, etc.) and have a title.

For ISO documents for microbiology of the food chain, the following annexes can be included (these are examples):

**Annex A**  
(normative)

**Flow diagram(s) of the procedure(s)**

Insert a flow diagram of the procedure(s), giving a schematic representation of the different steps of the procedure. The flow diagram constitutes a figure, to which a title has to be given. For example:

*Figure A.1 — Flow diagram of procedure for the detection of [microorganism]*

**Annex B**  
(normative)

**Culture media and reagents**

Give the composition and preparation of all culture media and reagents mentioned in the procedure.

The Chemical Abstracts Service (CAS) number of a chemical substance should be stated in the formulation. The CAS Number/CAS registry number is a unique numerical identifier of the Chemical Abstracts Service (CAS) for chemical elements, compounds, polymers, biological sequences, mixtures and alloys (ISO 11133:2014, 4.3.2).

CAS numbers are unique numerical identifiers assigned by the Chemical Abstracts Service. The CAS Registry Number is a registered trademark and therefore needs a disclaimer footnote, on the basis that ISO should not be seen to promote the “CAS Registry” product over other potential registries.
The ® symbol and footnote only need to be added to the first citation. There is no need to add them each time a CAS number is given.

For example:

**WARNING** — Hydrochloric acid (CAS Registry Number®¹ 7647-01-0) solution is toxic, corrosive, irritating and very toxic to aquatic life. Refer to the safety data sheet for details. Handling of hydrochloric acid solution shall be restricted to skilled personnel or conducted under their control. Care shall be taken in the disposal of this solution.

**WARNING** — Sodium hydroxide (CAS 1310-73-2) solution is toxic, corrosive and irritating. Refer to the safety data sheet for details. Handling of sodium hydroxide solution shall be restricted to skilled personnel or conducted under their control. Care shall be taken in the disposal of this solution.

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¹ CAS Registry Number® is a trademark of CAS corporation. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

For the description of the various components of culture media, consult ISO 11133:2014, Annex A. When the type of ‘peptone’ giving the best results is not known, the following comment should be added with an example.

Peptone

° For example, enzymatic digest of casein.

Give each culture medium or reagent a separate number to facilitate cross-referencing in the (full) text.

A general statement should be made in Clause B.1, stating:

*The general specifications of ISO 11133 are applicable to the preparation and performance testing of the culture media described in this annex. If culture media or reagents are prepared from dehydrated complete media/reagents or if ready-to-use media/reagents are used, follow the manufacturer’s instructions regarding preparation, storage conditions, expiry date and use.*

*The shelf life of the media indicated in this annex has been determined in some studies. The user shall verify this under its own storage conditions (in accordance with ISO 11133).*

*Performance testing of culture media is described in Clause B.#.*


Microbiological performance requirements (performance criteria, control method and targets) applicable to standardized culture media shall be included in each ISO document on microbiological analysis.
These performance specifications may be:

— imported from ISO 11133 for the culture media described therein or the specific ISO document published subsequently to ISO 11133; or

— after consultation with ISO/TC 34/SC 9/JWG 5, *Culture media*:
   
   — revised from ISO 11133 or the specific ISO document published subsequently to ISO 11133; or
   
   — created, for any new culture medium, in accordance with the principles defined in ISO 11133:2014, Annex J.

When drafting or revising an ISO document, strains included in the WDCM catalogue should be used in preference to other strains. Give preference to Risk Class 1 control strains for performance testing of culture media required for growth/isolation of non-pathogenic organisms.

When a new test strain is needed (in accordance with ISO 11133:2014, Clause J.4), a new WDCM number can be created (provided that the strain is available in two different culture collections). For this, the ISO/TC 34/SC 9 Committee Manager should be consulted.

The shortest permissible incubation time for productivity testing (target strains) should be specified and the longest permissible incubation time for selectivity testing (non-target strains).

Preferably, the same footnotes should be kept as given in ISO 11133:2014, Table E.1. If possible, this should at least be done for footnotes b and d.
## Table B.1 — Performance testing for the quality assurance of the culture media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Function</th>
<th>Incubation</th>
<th>Control strains</th>
<th>WDCM numbers(^a)</th>
<th>Method of control</th>
<th>Criteria(^e)</th>
</tr>
</thead>
</table>
| Non-selective broth           | Productivity      | Time range/temperature range| Target strain \(x^{c,d}\)  
Target strain \(y^{c,d}\)  
+ non-target strain \(a^{d}\)  
+ non-target strain \(b^{d}\) | 00XXX  
00YYY | Qualitative          | Turbidity (1–2)                                                                 |
| Selective broth               | Productivity      | Time range/temperature range| Target strain \(x^{c,d}\)  
Target strain \(y^{c,d}\)  
+ non-target strain \(a^{d}\)  
+ non-target strain \(b^{d}\) | 00XXX  
00YYY  
00AAA or 00aaa  
00BBB | Qualitative          | > 10 characteristic colonies on selective agar medium                           |
| Selectivity                   | Time range/temperature range | Non-target strain \(a^{d}\) | 00AAA or 00aaa                                                                 | Qualitative         | Partial inhibition ≤ 100 colonies on non-selective agar medium                 |
| Non-selective isolation medium| Productivity      | Time range/temperature range| Target strain \(x^{c,d}\)  
Target strain \(y^{c,d}\) | 00XXX  
00YYY | Qualitative          | Good growth (2)                                                                |
| Selective isolation medium    | Productivity      | Time range/temperature range| Target strain \(x^{c,d}\)  
Target strain \(y^{c,d}\) | 00XXX  
00YYY | Qualitative          | Good growth (2) of typical colonies                                            |
| Selectivity                   | Time range/temperature range | Non-target strain \(a^{d}\) | 00AAA or 00aaa                                                                 | Qualitative         | Growth or partial inhibition (0–1) of yellow colonies                          |
| Specificity                   | Non-target strain \((similar to the target strain)\) | 00EEE or 00eee | Qualitative          | Total inhibition (0) on non-selective agar medium                              |
| Non-selective enumeration medium| Productivity      | Time range/temperature range| Target strain \(x^{b}\)  
Target strain \(y\) | 00XXX  
00YYY | Quantitative         | \(P_R \geq 0.7\)                                                              |
| Selective enumeration medium  | Productivity      | Time range/temperature range| Target strain \(v\)  
Target strain \(w\) | 00VVV or 00WWV | Quantitative         | \(P_R \geq 0.5\) or 0.7 (see ISO 11133)                                      |
| Selectivity                   | Time range/temperature range | Non-target strain \(a^{e}\) | 00AAA or 00aaa | Qualitative         | Total or partial inhibition No characteristic colonies                        |
| Specificity                   | Non-target strain \((similar to the target strain)\) | 00GGG | Qualitative         | Growth with no characteristic colonies showing partial characteristics only   |
| Confirmation medium or reagent| Confirmation / Identification | Shortest permissible incubation time | Target strain \(u\) | 00UUU | Qualitative         | Positive reaction                                                             |
|                               |                    | Longest permissible incubation time | Non-target strain \(h\) | 00HHH | Qualitative         | Negative reaction                                                            |

\(^{a}\) Refer to the reference strain catalogue on [http://www.wfcc.info](http://www.wfcc.info) for information on culture collection strain numbers and contact details; WDCM: World Data Centre for Microorganisms.

\(^{b}\) Strains to be used as a minimum.

\(^{c}\) Some national restrictions and directions can require the use of a different serovar. Make reference to national requirements relating to the choice of the serovar(s).

\(^{d}\) Strain free of choice; one of the strains has to be used as a minimum.

\(^{e}\) Growth is categorized as 0: no growth; 1: weak growth (partial inhibition); 2: good growth (see ISO 11133).
Annex C
(informative)

Performance characteristics of the method

Tabulate in this annex the performance characteristics of the method as obtained from one or more interlaboratory studies.

For qualitative culture methods, provide information based on the following:

An interlaboratory study involving [number] laboratories in [number] countries was carried out. The following (food) items, [representing the (food) categories as indicated], were included in the study [specify the items tested and indicate the corresponding (food) category]. The (food) items were each tested at two [or another number] different levels of contamination, plus an uninoculated sample (negative control). The study was organized in [year] by [specify the organizing organization] as part of [specify the project].

The method submitted to the interlaboratory study was that of this document [specify ISO document number and year of publication if another edition/amendment of the ISO document or another ISO document was used] including the following [operational details; only necessary if another ISO edition/amendment/document was used].

Data obtained by some collaborators have been excluded from the calculations only on the basis of clearly identified technical reasons (e.g. deviations from the protocol).

The values of the performance characteristics, for each (food) item and category, derived from this interlaboratory study are shown in Tables C.1 to C.#, and were calculated in accordance with ISO 17468.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Blank</th>
<th>Low contamination level (# cfu/test portion&lt;sup&gt;a&lt;/sup&gt;)</th>
<th>High contamination level (# cfu/test portion&lt;sup&gt;a&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participating collaborators</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of samples per collaborator</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of collaborators retained after evaluation of data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of samples retained after evaluation of the data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test portion size, in g or ml</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Specificity, in %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity, in %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOD&lt;sub&gt;50&lt;/sub&gt; (95% confidence interval), in cfu/test portion</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* *Indicate the level of contamination of the samples tested. If the level of contamination is determined with an MPN method, the LOD<sub>50</sub> result is still expressed in cfu/test portion and not in MPN/test portion.

**NOTE** Strain used for inoculation: [name and identification of the strain].
For **quantitative culture methods**, provide information based on the following:

An interlaboratory study involving [number] laboratories in [number] countries was carried out. The following (food) items, [representing the (food) categories as indicated], were included in the study [specify the items tested and indicate the corresponding (food) category]. The (food) items were each tested at three [or another number] different levels of contamination. The study was organized in [year] by the [specify the organizing organization] as part of [specify the project].

The method submitted to the interlaboratory study was that of this document [specify ISO document number and year of publication if another edition/amendment of the ISO document or another ISO document was used] including the following [operational details; only necessary if another ISO edition/amendment/document was used].

Data obtained by some collaborators have been excluded from the calculations only on the basis of clearly identified technical reasons (e.g. deviations from the protocol).

The values of the performance characteristics, for each (food) item and category, derived from this interlaboratory study are shown in Tables C.1 to C.#, and were calculated in accordance with ISO 17468.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Contamination level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Number of participating collaborators</td>
<td></td>
</tr>
<tr>
<td>Number of collaborators retained after evaluation of the data</td>
<td></td>
</tr>
<tr>
<td>Number of samples</td>
<td></td>
</tr>
<tr>
<td>Number of sample results retained after evaluation of the data</td>
<td></td>
</tr>
<tr>
<td>Mean value $\Sigma a \ (\log_{10} \text{cfu/g})$</td>
<td></td>
</tr>
<tr>
<td>Interlaboratory repeatability standard deviation, $s_i \ (\log_{10} \text{cfu/g})$</td>
<td></td>
</tr>
<tr>
<td>Interlaboratory reproducibility standard deviation, $s_R \ (\log_{10} \text{cfu/g})$</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE** Strain used for inoculation: [name and identification of the strain].

For **confirmation or typing methods**, provide information based on the following:

An interlaboratory study involving [number] laboratories in [number] countries was carried out. The following strains were included in the study: [specify the strains tested]. The study was organized in [year] by the [specify the organizing organization] as part of [specify the project].

The method submitted to the interlaboratory study was that of this document [specify ISO document number and year of publication if another edition/amendment of the ISO document or another ISO document was used] including the following [operational details; only necessary if another ISO edition/amendment/document was used].

Data obtained by some collaborators have been excluded from the calculations only on the basis of clearly identified technical reasons (e.g. deviations from the protocol).

The values of the performance characteristics, derived from this interlaboratory study are shown in Tables C.1 to C.#, and were determined in accordance with ISO 17468.
Table C.1 — Details interlaboratory study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participating collaborators</td>
<td></td>
</tr>
<tr>
<td>Samples per collaborator</td>
<td></td>
</tr>
<tr>
<td>Collaborators retained after evaluation of data</td>
<td></td>
</tr>
<tr>
<td>Samples retained after evaluation of the data</td>
<td></td>
</tr>
</tbody>
</table>

Table C.2 — Inclusivity and exclusivity data from the interlaboratory study

<table>
<thead>
<tr>
<th>Performance characteristic</th>
<th>Number of different strains</th>
<th>Total number of results</th>
<th>Inclusivity agreement</th>
<th>Inclusivity deviation</th>
<th>Exclusivity agreement</th>
<th>Exclusivity deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inclusivity</td>
<td></td>
<td></td>
<td></td>
<td>Not applicable</td>
<td>Not applicable</td>
<td></td>
</tr>
<tr>
<td>Exclusivity</td>
<td></td>
<td></td>
<td>Not applicable</td>
<td>Not applicable</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In case of inclusivity deviation(s) and/or exclusivity deviation(s), an explanation should be provided below Table C.2.

**Bibliography**

The Bibliography lists, for information, those documents which are cited informatively in the document. Other background documents may be included if deemed useful for the user. The documents are numbered consecutively and this number is included in the text (except for ISO documents). References to documents in the Bibliography can be cited in the text in a sentence, e.g. ‘in Reference [17]’ or presented in superscript, e.g. ‘...microbiology of the food chain[17].’

In the Bibliography, start with ISO documents listed in ascending numerical order, followed by other resources (printed, electronic, etc.). For references to books and publications, the relevant rules set out in ISO 690 shall be followed.
Guidance Document, 3rd edition

EXAMPLE: Annex 1 (informative) Examples for the content of clauses of an International Standard for a qualitative microbiological culture method

Annex 1 provides examples of what may be written in clauses or subclauses of an International Standard for a qualitative culture method, in addition to the 'general situation' or 'standard sentences' as described in the main text of this Guidance Document.

NOTE 1 This Guidance Document is using Annex 1, Annex 2, etc., to distinguish from Annex A, Annex B, etc. as commonly used in International Standards.

NOTE 2 Annex 1 might differ from the text in ISO 6579-1:2017, because the text has been modified to be consistent with the main text of the Guidance Document.

ISO 6579-1, Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp.

Foreword

['Standard sentences’ are included by ISO Central Secretariat]

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

This first edition cancels and replaces ISO 6579:2002 and ISO 6785:2001, which have been technically revised. It also incorporates the Amendment ISO 6579:2002/Amd.1:2007 and the Technical Corrigendum ISO 6579:2002/Cor.1:2004. The main changes are as follows.

— samples from the primary production stage have been added to the Scope;
— the detection of Salmonella Typhi and Salmonella Paratyphi has been added to Annex D.
— [.....]

Introduction

This document describes a horizontal method for the detection of Salmonella spp. in food (including milk and milk products, originally described in ISO 6785), in animal feed, in animal faeces and in environmental samples from the primary production stage (the latter two were originally described in ISO 6579:2002/Amd.1:2007). The main technical changes listed in the Foreword, introduced in this document compared with ISO 6579:2002, are considered as minor (see ISO 17468). These technical changes have a minor impact on the performance characteristics of the method.

A procedure for the enumeration of Salmonella spp. is described in ISO/TS 6579-2.

Guidance for serotyping of Salmonella spp. is described in ISO/TR 6579-3.

1 Scope

This document specifies a horizontal method for the detection of Salmonella.
It is applicable to the following: [include ‘standard sentences’, see main text of this Guidance Document].

NOTE This method has been validated in interlaboratory studies for the following food categories:

— raw milk and dairy products;
— heat-processed milk and dairy products;
— raw poultry and ready-to-cook poultry products;
— eggs and egg products (derivates).

It has also been validated for the following other category:

— primary production samples (PPS).

For detailed information on the validation see Clause 11 and Annex C.

Example of description of the method limitation:

This horizontal method was originally developed for the examination of all samples belonging to the food chain. However, because of the large variety of products in the food chain, it is possible that this horizontal method is not appropriate in every detail for all products. Nevertheless, it is expected that the required modifications are minimized so that they do not result in a significant deviation from this horizontal method.

Based on the information available at the time of publication of this document, this method is not considered to be (fully) suited to the examination of non-motile Salmonella in samples from the primary production stage (PPS). The selective enrichment medium modified semi-solid Rappaport-Vassiliadis (MSRV) agar, is intended for the detection of motile Salmonella and is not appropriate for the detection of non-motile Salmonella strains.

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

3 Terms and definitions

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

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

— ISO Online browsing platform: available at https://www.iso.org/obp
— IEC Electropedia: available at https://www.electropedia.org/
3.1 **Salmonella**

Genus of microorganisms of the family *Enterobacteriaceae*, usually capable of growth in/on Rappaport-Vassiliadis medium with soya (RVS) broth, modified semi-solid Rappaport-Vassiliadis (MSRV) agar and Muller-Kauffmann tetrathionate-novobiocin (MKTTn) broth, forming typical or less typical colonies on solid selective media, such as xylose lysine deoxycholate (XLD) agar, and displaying certain characteristics with biochemical and serological confirmation tests.

Note 1 to entry: The biochemical and serological confirmation tests and the characteristics of *Salmonella* are described in 9.5.

3.2 Detection of *Salmonella*

Determination of *Salmonella* (3.1), in a particular mass or volume of product or surface area or object (e.g. boot socks), when specified tests are carried out.

Note 1 to entry: Specified tests are given in Clause 9.

### 4 Principle

4.1 General

The detection of *Salmonella* requires four successive steps as specified in Annex A.

4.2 Pre-enrichment in non-selective liquid medium

Buffered peptone water at ambient temperature is inoculated with the test portion, then incubated between 34 °C and 38 °C for 18 h.

4.3 Enrichment in/on selective media

Rappaport-Vassiliadis medium with soya (RVS broth) or modified semi-solid Rappaport-Vassiliadis (MSRV) agar and Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn broth) are inoculated with the culture obtained in 4.2.

The RVS broth or the MSRV agar is incubated at 41.5 °C for 24 h and the MKTTn broth between 34 °C and 38 °C for 24 h.

### 5 Culture media and reagents

[Include ‘standard sentences’, see main text of this Guidance Document]

### 6 Equipment and consumables

[Include ‘standard sentences’, see main text of this Guidance Document]

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

6.2 Drying cabinet or oven, capable of operating between 25 °C and 50 °C.

6.3 Incubator, capable of operating in the range 34 °C to 38 °C.

NOTE The range 34 °C to 38 °C for incubation of culture media includes the use of incubators set at 35 °C ± 1 °C, 36 °C ± 2 °C or 37 °C ± 1 °C.
6.4 **Incubator**, capable of operating at 41.5 °C ± 1 °C, or **water bath**, capable of operating at 41.5 °C ± 1 °C.

It is recommended to use a water bath containing an antibacterial agent because of the low infective dose of *Salmonella*.

6.5 **Refrigerator**, capable of operating at 5 °C ± 3 °C.

6.6 **Sterile loops**, of approximate diameter 3 mm (10 µl volume) and of 1 µl volume, and **inoculation needle** or **wire**.

6.7 **Sterile tubes, bottles or flasks** with caps, of appropriate capacity.

Bottles or flasks with non-toxic metallic or plastic screwcaps may be used.

6.8 **Sterile graduated pipettes** or **automatic pipettes**, of nominal capacities 25 ml, 10 ml, 1 ml and 0.1 ml.

6.9 **Sterile Petri dishes**, with a diameter of approximately 90 mm and (optional) large size (diameter of approximately 140 mm).

[......]

7 **Sampling**

*[Include 'standard sentences', see main text of this Guidance Document]*

8 **Preparation of test sample**

*[Include 'standard sentences', see main text of this Guidance Document]*

9 **Procedure**

9.1 **Test portion and initial suspension**

For preparation of the initial suspension, in the general case, use as a diluent the pre-enrichment medium specified in Clause B.1: buffered peptone water (BPW). Pre-warm the BPW to room temperature before use.

In general, an amount of test portion (mass or volume) is added to a quantity of BPW (mass or volume) to yield a tenfold dilution; for this, a 25 g test portion is mixed with 225 ml of BPW. However, for some type of samples (e.g. boot socks, dust) it can be necessary to use another ratio.

For specific products, follow the procedures specified in the ISO 6887 series.

This document has been validated for test portions of 25 g. *[Include 'standard sentences' for size of test portion, see main text of this Guidance Document]*.

[......]

9.2 **Non-selective pre-enrichment**

Incubate the initial suspension obtained in 9.1 between 34 °C and 38 °C (6.3) for 18 h ± 2 h.
It is permissible to store the pre-enriched sample after incubation at 5 °C (6.5) for a maximum of 72 h (see References [#] to [#]).

9.3 Selective enrichment

9.3.1 General

Allow the selective enrichment media, RVS or MSRV (Clause B.3 or Clause B.4) and MKTTn (Clause B.5), to equilibrate at room temperature if they were stored at a lower temperature.

Minimize the transfer of particulate material from the pre-enrichment into the selective enrichment media.

After incubation, it is permissible to store the selective enrichment at 5 °C (6.5) for a maximum of 72 h (see References [#] to [#]).

NOTE MSRV agar is intended for the detection of motile Salmonella strains and is not appropriate for the detection of non-motile Salmonella strains.

9.3.2 Procedure for food, animal feed samples and environmental samples from the food production area

Transfer 0.1 ml of the culture obtained in 9.2 to a tube containing 10 ml of the RVS broth (Clause B.3) or to the surface of a MSRV-agar plate (Clause B.4). Inoculate the MSRV agar with one to three equally spaced spots on the surface of the medium.

Transfer 1 ml of the culture obtained in 9.2 to a tube containing 10 ml MKTTn broth (Clause B.5).

Incubate the inoculated RVS broth at 41.5 °C (6.4) for 24 h ± 3 h.

Incubate the inoculated MSRV-agar plates at 41.5 °C (6.4) for 24 h ± 3 h. Do not invert the plates.

Incubate the inoculated MKTTn broth between 34 °C and 38 °C (6.3) for 24 h ± 3 h.

Suspect MSRV plates will show a grey-white, turbid zone extending out from the inoculated drop.

In dried milk products and cheese, Salmonella can be sublethally injured. Incubate the selective enrichment media from these products for an additional 24 h ± 3 h (see Reference [#]).

For some other products, e.g. when investigating outbreak samples, this additional incubation time can also be beneficial.

9.3.3 Procedure for samples from the primary production stage

[Similar layout as the text shown above]

9.4 Plating out and identification

9.4.1 General

[Similar layout as the text shown above]
9.4.2 Procedure for food, animal feed samples and environmental samples from the food production area

[Similar layout as the text shown above]

9.4.3 Procedure for samples from the primary production stage

[Similar layout as the text shown above]

9.5 Confirmation

9.5.1 General

The combination of biochemical and serological test results indicates whether an isolate belongs to the genus *Salmonella*. For characterization of *Salmonella* strains, full serotyping is needed. Guidance for serotyping is described in ISO/TR 6579-3.

For some of the confirmation media, as specified in 9.5.3 and in Clauses B.# to B.#, alternative (commercial) formulations exist which can also be applicable for biochemical confirmation of *Salmonella*. These alternative formulations are allowed, provided that the performance for the biochemical confirmation of *Salmonella* is verified before use.

For a clear distinction between positive and negative biochemical reactions, it is helpful to verify the reactions of the media of each biochemical test with well-characterized positive and negative control strains.

NOTE 1 The recognition of colonies of *Salmonella* is, to a large extent, a matter of experience and their appearance can vary somewhat, not only from serovar to serovar, but also from batch to batch of the selective culture medium used.

If shown to be reliable, miniaturized galleries for the biochemical identification of *Salmonella* may be used (see ISO 7218).

NOTE 2 Alternative procedures (see ISO 7218) can be used to confirm the isolate as *Salmonella* spp., providing the suitability of the alternative procedure is validated (see ISO 16140-4 or ISO 16140-6).

9.5.2 Selection of colonies for confirmation

Mark suspect colonies on each plate obtained in 9.4. Select at least one suspect colony for subculture and confirmation. If this is negative, select up to four more suspect colonies, ensuring that these colonies are subcultured from different selective enrichment/isolation medium combinations showing suspect growth.

NOTE For epidemiological purposes or during outbreak investigations, confirmation of additional colonies, e.g. five typical or suspect colonies from each selective enrichment/isolation medium combination, can be beneficial.

[......]

10 Expression of results

[Include 'standard sentences', see main text of this Guidance Document]
Validation of the method

11.1 Validation in accordance with ISO 17468

[Include ‘standard sentences’, see main text of this Guidance Document]

11.2 Performance characteristics

[Include ‘standard sentences’, see main text of this Guidance Document]

A summary of the LOD\(_{50}\) values is given in Table 1.

<table>
<thead>
<tr>
<th>(Food) category</th>
<th>(Food) item</th>
<th>LOD(_{50}) in cfu/test portion</th>
<th>Test portion size</th>
<th>Salmonella serovar used in the interlaboratory study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw milk and dairy products</td>
<td>Fresh cheese curd</td>
<td>5,7</td>
<td>25 g</td>
<td>Montevideo</td>
</tr>
<tr>
<td>Raw poultry and ready-to-cook poultry products</td>
<td>Raw poultry meat</td>
<td>2,2</td>
<td>25 g</td>
<td>Naturally contaminated</td>
</tr>
<tr>
<td>Eggs and eggs products (derivates)</td>
<td>Dried egg powder</td>
<td>6</td>
<td>25 g</td>
<td>Panama</td>
</tr>
<tr>
<td>Primary production samples (PPS)</td>
<td>Chicken faeces</td>
<td>1</td>
<td>10 g</td>
<td>Typhimurium</td>
</tr>
<tr>
<td>Primary production samples (PPS)</td>
<td>Chicken faeces</td>
<td>4,3</td>
<td>10 g</td>
<td>Enteritidis</td>
</tr>
<tr>
<td>Primary production samples (PPS)</td>
<td>Chicken faeces</td>
<td>2,5</td>
<td>10 g</td>
<td>Combined (Typhimurium and Enteritidis)</td>
</tr>
<tr>
<td>Primary production samples (PPS)</td>
<td>Boot socks plus laying hen faeces</td>
<td>3,8</td>
<td>10 g in boot sock</td>
<td>Typhimurium</td>
</tr>
<tr>
<td>Primary production samples (PPS)</td>
<td>Pig faeces</td>
<td>2,8</td>
<td>25 g</td>
<td>Derby</td>
</tr>
<tr>
<td>Primary production samples (PPS)</td>
<td>Pig faeces</td>
<td>3,8</td>
<td>25 g</td>
<td>Typhimurium</td>
</tr>
<tr>
<td>Primary production samples (PPS)</td>
<td>Pig faeces</td>
<td>3,2</td>
<td>25 g</td>
<td>Combined (Derby and Typhimurium)</td>
</tr>
</tbody>
</table>

Test report

[Include ‘standard sentences’, see main text of this Guidance Document]

Quality assurance

[Include ‘standard sentences’, see main text of this Guidance Document]
Annex A
(normative)

Flow diagram of the procedure

Figure A.1 — Flow diagram of procedure for detection of Salmonella in food, animal feed and environmental samples from the food production area
Annex B
(normative)

Culture media and reagents

B.1 General

[Include ‘standard sentences’, see main text of this Guidance Document]

B.2 Buffered peptone water (BPW)

B.2.1 Composition

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone(^a)</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Sodium chloride (NaCl)</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate dodecahydrate (Na(_2)HPO(_4)·12(\text{H}_2\text{O}))(^b)</td>
<td>9.0 g</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate (KH(_2)PO(_4))</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

\(^a\) For example, enzymatic digest of casein.

\(^b\) If disodium hydrogen phosphate with a different water content is used, amend the mass of the ingredient accordingly. For example, in the case of anhydrous disodium hydrogen phosphate (Na\(_2\)HPO\(_4\)), (CAS No. 7558-79-4), use 3.57 g.

B.2.2 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 20°C to 25°C.

Dispense the medium into flasks (6.7) 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 containers at 5°C (6.5) for up to six months.

B.3 Rappaport-Vassiliadis medium with soya (RVS broth)

B.3.1 Solution A

B.3.1.1 Composition

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatic digest of soya</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Sodium chloride (NaCl)</td>
<td>8.0 g</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate (KH(_2)PO(_4))</td>
<td>1.4 g</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate (K(_2)HPO(_4))</td>
<td>0.2 g</td>
</tr>
<tr>
<td>Water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

\(^1\) CAS Registry Number® is a trademark of CAS corporation. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.
B.3.1.2 Preparation
Dissolve the components in the water by heating to about 70 °C, if necessary.
The solution shall be prepared on the day of preparation of the complete RVS medium.

B.3.2 Solution B
B.3.2.1 Composition

<table>
<thead>
<tr>
<th>Component</th>
<th>Mass (g)</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium chloride hexahydrate (MgCl$_2$$\cdot$6H$_2$O) (CAS No. 7791-18-6)</td>
<td>400.0</td>
<td>1 000</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B.3.2.2 Preparation
Dissolve the magnesium chloride in the water.

As this salt is very hygroscopic, it is advisable to dissolve the entire contents of MgCl$_2$$\cdot$6H$_2$O from a newly opened container in accordance with the formula. For instance, 250 g of MgCl$_2$$\cdot$6H$_2$O is added to 625 ml of water giving a solution of total volume of 788 ml and a mass concentration of about 31.7 g per 100 ml of MgCl$_2$$\cdot$6H$_2$O.
The solution may be kept in a dark glass bottle with tight stopper at room temperature for at least two years.

B.3.3 Solution C
B.3.3.1 Composition

<table>
<thead>
<tr>
<th>Component</th>
<th>Mass (g)</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malachite green oxalate (CAS No. 2437-29-8)</td>
<td>0.4</td>
<td>100</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B.3.3.2 Preparation
Dissolve the malachite green oxalate in the water.
The solution may be kept in a brown glass bottle at room temperature for at least eight months.

B.3.4 Complete medium
B.3.4.1 Composition

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution A (B.3.1)</td>
<td>1 000</td>
</tr>
<tr>
<td>Solution B (B.3.2)</td>
<td>100</td>
</tr>
<tr>
<td>Solution C (B.3.3)</td>
<td>10</td>
</tr>
</tbody>
</table>

B.3.4.2 Preparation
Add to 1 000 ml of solution A, 100 ml of solution B and 10 ml of solution C.
Adjust the pH, if necessary, so that after sterilization it is 5.2 ± 0.2 at 20 °C to 25 °C.
Dispense the medium into tubes or flasks (6.7) of suitable capacity to obtain the portions necessary for the test, e.g. 10 ml quantities dispensed into tubes.
Sterilize for 15 min in the autoclave (6.1) set at 115 °C.
Store the complete medium in closed tubes or flasks at 5 °C (6.5) for up to three months.
NOTE The final medium composition is enzymatic digest of soya 4.5 g/l, sodium chloride (NaCl) 7.2 g/l, potassium dihydrogen phosphate (KH₂PO₄ + K₂HPO₄) 1.44 g/l, anhydrous magnesium chloride (MgCl₂) (CAS No. 7786-30-3) 13.4 g/l, or magnesium chloride hexahydrate (MgCl₂·6H₂O) (CAS No. 7791-18-6) 28.6 g/l, and malachite green oxalate 0.036 g/l.

B.4 Performance testing

The definitions of productivity, selectivity and specificity are specified in ISO 11133. Table B.1 describes the performance testing of the different culture media. For performance testing of selective liquid media and MSRV agar, use the same inoculum volume as specified in 9.3.2. For MSRV agar, the inoculum should contain 10³ cfu to 10⁴ cfu for determining productivity and specificity, and 10⁴ cfu to 10⁶ cfu for determining selectivity (see ISO 11133). For the other media, the inoculum levels for the target and the non-target microorganisms are specified in ISO 11133:2014, 5.4. For confirmation media, refer to ISO 11133:2014/Amd.2:2020, Annex K.
Table B.1 — Performance testing for the quality assurance of the culture media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Function</th>
<th>Incubation</th>
<th>Control strains</th>
<th>WDCM numbers①</th>
<th>Method of control</th>
<th>Criteria②</th>
</tr>
</thead>
</table>
| BPW            | Productivity | 18 h ± 2 h / 34 °C to 38 °C | *Salmonella Typhimurium*<sup>cd</sup>  
*Salmonella Enteritidis*<sup>cd</sup> | 00031  
00030 | Qualitative | Turbidity (1–2) |
| MKTTn broth    | Productivity | 24 h ± 3 h / 34 °C to 38 °C | *Salmonella Typhimurium*<sup>cd</sup>  
*Salmonella Enteritidis*<sup>cd</sup>  
+ *Escherichia coli*  
+ *Pseudomonas aeruginosa* | 00031  
00030  
00012 or  
00013  
00025 | Qualitative | > 10 characteristic colonies on XLD or other medium of choice |
|                | Selectivity |                          | *Escherichia coli*<sup>a</sup>  
*Enterococcus faecalis*<sup>a</sup> | 00012 or  
00013  
00009 or  
00087 | Qualitative | Partial inhibition ≤ 100 colonies on TSA |
|                |            |                            |                                          |                | Qualitative | < 10 colonies on TSA |
| MSRV agar      | Productivity | 2 × (24 h ± 3 h) / 41.5 °C ± 1 °C | *Salmonella Typhimurium*<sup>cd</sup>  
*Salmonella Enteritidis*<sup>cd</sup> | 00031  
00030 | Qualitative | Grey-white, turbid zone extending out from inoculated drop(s). After 24 h to 48 h, the turbid zone(s) will be (almost) fully migrated over the plate. Possible extra: characteristic colonies after subculturing on XLD |
|                | Selectivity |                            | *Escherichia coli*<sup>a</sup>  
*Enterococcus faecalis*<sup>a</sup> | 00012 or  
00013  
00009 or  
00087 | Qualitative | Possible growth at the place of the inoculated drop(s) without a turbid zone |
|                |            |                            |                                          |                | Qualitative | No growth |
| XLD agar       | Productivity | 24 h ± 3 h / 34 °C to 38 °C | *Salmonella Typhimurium*<sup>cd</sup>  
*Salmonella Enteritidis*<sup>cd</sup> | 00031  
00030 | Qualitative | Good growth (2) of colonies with black centre and a lightly transparent zone of reddish colour due to the colour change of the medium |
|                | Selectivity |                            | *Escherichia coli*<sup>a</sup>  
*Enterococcus faecalis*<sup>a</sup> | 00012 or  
00013  
00009 or  
00087 | Qualitative | Growth or partial inhibition (0 to 1) of yellow colonies |
|                | Specificity |                            | *Escherichia coli*<sup>a</sup> | 00012 or  
00013 | Qualitative | Total inhibition (0) |
| LDC medium     | Detection of L-Lysine decarboxylase (LDC) | 24 ± 3 h / 34 °C to 38 °C | *Salmonella Typhimurium*<sup>cd</sup>  
*Salmonella Enteritidis*<sup>cd</sup> | 00031  
00030 | Qualitative | Positive reaction: Medium remains purple after incubation and is turbid |
|                |            |                            | *Escherichia coli* | 00012 or  
00013 | Qualitative | Negative reaction: Medium changes from purple to yellow |

① Refer to the reference strain catalogue on [http://www.wfcc.info](http://www.wfcc.info) for information on culture collection strain numbers and contact details; WDCM: World Data Centre for Microorganisms.

② Growth is categorized as: 0: no growth; 1: weak growth (partial inhibition); 2: good growth (see ISO 11133).

③ Some national restrictions and directions can require the use of a different serovar. Make reference to national requirements relating to the choice of *Salmonella* serovars.

④ Strain free of choice; one of the strains has to be used as a minimum.
Annex C
(informative)

Performance characteristics of the method

C.1 Performance characteristics of MSRV for detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage

The precision data of MSRV for detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage were calculated from three different interlaboratory studies organized by the EURL-*Salmonella*, RIVM, the Netherlands. These studies were organized in 2008[^1], 2012[^2] and 2013[^3]. The samples tested in the three studies were chicken faeces, pig faeces and boot socks, respectively. The samples were each tested at two different levels of contamination, plus an uninoculated sample (negative control). All studies were funded by the European Commission and the latter study was also performed as part of the CEN Mandate M381.

The method submitted to the interlaboratory studies was that of ISO 6579:2002/Amd.1:2007[^4] for the detection of *Salmonella* in samples from the primary production stage, including selective enrichment on MSRV. This method has been incorporated in this document.

Data obtained by some collaborators have been excluded from the calculations only on the basis of clearly identified technical reasons (deviations to the protocol).

The values of the performance characteristics, for each (food) item and category, derived from the interlaboratory studies are shown in Tables C.1 to C.#, and were calculated in accordance with ISO 17468.

**Table C.1 — Results of data analysis obtained with pig faeces samples [category: primary production samples (PPS)]**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participating collaborators</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Number of samples per collaborator</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Number of collaborators retained after evaluation of data</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Number of samples retained after evaluation of data</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>130</td>
</tr>
<tr>
<td>Test portion size, in g</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Specificity, in %</td>
<td>99,2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sensitivity per serovar and level, in %</td>
<td>—</td>
<td>88,5</td>
<td>97,7</td>
<td>91,5</td>
<td>98,5</td>
</tr>
<tr>
<td>LOD[^9] per serovar (95 % confidence interval), in cfu/test portion</td>
<td>—</td>
<td>2,8 (2,2 to 3,5)</td>
<td>3,8 (3,0 to 4,7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOD[^9] overall (95 % confidence interval), in cfu/test portion</td>
<td>—</td>
<td>3,2 (2,8 to 3,8)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[^1]: Pig faeces samples were artificially contaminated with reference materials with the following strains and levels:
— *Salmonella* Derby (SD) at a level of 6 cfu/test portion and a level of 37 cfu/test portion (NCTC 5722);
— *Salmonella* Typhimurium (STM) at a level of 10 cfu/test portion and a level of 58 cfu/test portion (WDCM 00031).
EXAMPLE: Annex 2 (informative) Examples for the content of clauses of an International Standard for a quantitative microbiological culture method

Annex 2 provides examples of what may be written in clauses or subclauses of an International Standard for a quantitative culture method, in addition to the ‘general situation’ or ‘standard sentences’ as described in the main text of this Guidance Document.

NOTE 1 In International Standards, the second annex is numbered as ‘Annex B’ instead of ‘Annex 2’.

NOTE 2 Annex 2 might differ from the text in ISO 10272-2:2017, because the text has been modified to be consistent with the main text of the Guidance Document.

ISO 10272-2, Microbiology of the food chain — Horizontal method for the detection and enumeration of Campylobacter spp. — Part 2: Colony-count technique

1 Scope

[See example Annex 1 of this Guidance Document]

2 Normative references

[See example Annex 1 of this Guidance Document]

3 Terms, definitions and abbreviated terms

3.1 Terms and definitions

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

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

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

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

3.1.1 Campylobacter

genus of microorganisms of the family Campylobacteraceae, forming characteristic colonies on solid selective media, such as modified charcoal cefoperazone deoxycholate agar (mCCD agar), when incubated in a microaerobic atmosphere at 41.5 °C and displaying certain characteristics with biochemical confirmation tests and by microscopy

Note 1 to entry: Microscopy and the biochemical confirmation tests and the characteristics of Campylobacter are described in 9.4.

Note 2 to entry: This document targets the thermotolerant Campylobacter species relevant to human health. The most frequently encountered and relevant to human health are Campylobacter jejuni and Campylobacter coli. However, other species have been described (Campylobacter lari, Campylobacter upsaliensis and others).

3.1.2 enumeration of Campylobacter
determination of the number of colony-forming units (cfu) of Campylobacter (3.1) found per gram, per millilitre, per square centimetre or per sampling device when a specified test is conducted

Note 1 to entry: Specified tests are given in Clause 9.
3.2 Abbreviated terms

cfu colony-forming units
MALDI-TOF matrix-assisted laser desorption/ionization time-of-flight
WDCM World Data Centre for Microorganisms

4 Principle

4.1 General

The enumeration of *Campylobacter* requires three successive steps as specified in Annex A.

4.2 Preparation of dilutions

For the preparation of decimal dilutions from the test portion, see the ISO 6887 series.

4.3 Enumeration

The solid selective medium, modified charcoal cefoperazone deoxycholate agar (mCCD agar), is inoculated with a specified quantity of the test portion if the product is liquid, or of the initial suspension in the case of other products.

Other plates are prepared under the same conditions, using decimal dilutions of the test portion or of the initial suspension.

The plates are incubated at 41.5 °C in a microaerobic atmosphere and examined after 44 h to record the number of suspect *Campylobacter* colonies.

4.4 Confirmation

[Similar layout as the text shown above]

5 Culture media and reagents

[See example Annex 1 of this Guidance Document]

6 Equipment and consumables

[See example Annex 1 of this Guidance Document]

Additional example for microaerobic incubation:

6.10 **Appropriate apparatus for achieving a microaerobic atmosphere**, with oxygen content of 5 % ± 2 %, carbon dioxide 10 % ± 3 %, optional hydrogen ≤ 10 %, with the balance nitrogen.

The appropriate microaerobic atmosphere can be obtained using gas-tight jars and gas-generating kits, following precisely the manufacturer’s instructions. Alternatively, the jar or incubator may be filled with an appropriate gas mixture prior to incubation.
7 Sampling

[Include ‘standard sentences’, see main text of this Guidance Document]

Since *Campylobacter* is very sensitive to freezing but survives best at low temperatures, samples to be tested should not be frozen, but stored at 3 °C (6.°) and subjected to analysis as rapidly as possible. In addition, take care to prevent the samples from drying.

8 Preparation of test sample

[Include ‘standard sentences’, see main text of this Guidance Document]

9 Procedure

9.1 Test portion, initial suspension and dilutions

Follow the procedures in accordance with the ISO 6887 series and the specific International Standard dealing with the product concerned.

Prepare a single decimal dilution series from the test portion if the product is liquid, or from the initial suspension in the case of other products.

9.2 Inoculation and incubation

9.2.1 Using a sterile pipette, transfer 0,1 ml of the initial suspension (or sample if liquid) obtained in 9.1 to the mCCD-agar plate (Clause B.°). Repeat the procedure using further decimal dilutions if necessary. If only the initial suspension is used, also prepare duplicate plates using an additional agar plate.

When, for certain products, it is necessary to estimate low numbers of *Campylobacter*, the limit of enumeration may be lowered by a factor of 10 by examining 1,0 ml of the initial suspension. Distribute the 1,0 ml of inoculum either on the surface of the agar medium in a large Petri dish (140 mm) or three regular Petri dishes (90 mm). In both cases, prepare duplicates by using two large plates or six regular plates.

9.2.2 Evenly spread the inoculum, as quickly as possible, over the surface of the agar plate, using a sterile spreader. Avoid touching the sides of the Petri dish.

NOTE Drying of the plates is critical to produce countable plates. Guidance for drying of the plates can be found in ISO 11133:2014, 4.5.5.

9.2.3 Incubate the plates obtained in 9.2.1 at 41,5 °C (6.°) in a microaerobic atmosphere (6.°).

9.3 Enumeration of characteristic colonies

9.3.1 After 44 h ± 4 h of incubation, examine the plates obtained in 9.2.3 for typical and/or suspect colonies of *Campylobacter*.

The typical colonies are greyish on mCCD agar, often with a metallic sheen, and are flat and moist, with a tendency to spread. Colonies spread less on drier agar surfaces. Other forms of colonies can occur.

NOTE The recognition of colonies of *Campylobacter* is to a large extent a matter of experience and their appearance can vary somewhat, not only from strain to strain, but also from batch to batch of the selective culture medium used.
9.3.2 Select the plates obtained in 9.3.1 containing less than 150 typical or suspect colonies. Count these colonies and record their number as presumptive colonies per dish. Then choose at random five such colonies for subculturing for the confirmation tests (see 9.4).

9.4 Confirmation of Campylobacter

9.4.1 General

As Campylobacter rapidly loses culturability in air, follow the procedure described in 9.4.2 to 9.4.5 without delay.

9.4.2 Selection of colonies for confirmation

9.4.2.1 For confirmation, take five presumptive colonies from each dish retained for enumeration (see 9.3.2).

9.4.2.2 Streak each of the selected colonies onto a non-selective blood agar plate, e.g. Columbia blood agar (Clause B.9) in order to allow the development of well-isolated colonies. Incubate the plates in a microaerobic atmosphere (6.9) at 41.5 °C (6.9) for 24 h to 48 h. Use well-isolated freshly grown colonies for examination of morphology and motility (see 9.4.3), absence of aerobic growth at 25 °C (see 9.4.4) and the presence of oxidase (see 9.4.5).

NOTE The suspect colony can be previewed for characteristic morphology and motility before streaked on blood agar.

9.4.6 Interpretation

Campylobacter gives results in accordance with Table 1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Typical for Campylobacter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology (9.4.3)</td>
<td>Small curved bacilli*</td>
</tr>
<tr>
<td>Motility (9.4.3)</td>
<td>Characteristic corckscrew darting*</td>
</tr>
<tr>
<td>Aerobic growth at 25 °C (9.4.4)</td>
<td>–</td>
</tr>
<tr>
<td>Oxidase (9.4.5)</td>
<td>+</td>
</tr>
</tbody>
</table>

Key
+ Positive.
– Negative.
* Older cultures can rapidly lose their characteristic shape and motility and turn into less motile cocoid-like forms.

9.5 Identification of Campylobacter species (optional)

[Similar layout as the text shown above]

10 Expression of results

For calculation of the results, follow the procedure(s) in accordance with ISO 7218. Calculate and report the results as the number of Campylobacter in cfu per gram, per millilitre or per square centimetre. When the sampled area is not known, report as per sampling device, such as a cloth, sponge swab or stick.
11 Validation of the method

11.1 Validation in accordance with ISO 17468

[Include 'standard sentences', see main text of this Guidance Document]

11.2 Performance characteristics

[Include 'standard sentences', see main text of this Guidance Document]

A summary of the interlaboratory repeatability standard deviations ($s_r$) is given in Table 2.

<table>
<thead>
<tr>
<th>(Food) category</th>
<th>(Food) item</th>
<th>$s_r$ values from the interlaboratory study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low inoculation level</td>
</tr>
<tr>
<td>Raw milk and dairy products</td>
<td>Raw milk</td>
<td>0,19</td>
</tr>
<tr>
<td>Raw meat and ready-to-cook meat products (except poultry)</td>
<td>Minced meat</td>
<td>0,17</td>
</tr>
<tr>
<td>Raw poultry and ready-to-cook poultry products</td>
<td>Chicken skin</td>
<td>0,17</td>
</tr>
<tr>
<td>Processed fruits and vegetables</td>
<td>Frozen spinach</td>
<td>0,17</td>
</tr>
<tr>
<td>Primary production samples (PPS)</td>
<td>Broiler caecal material</td>
<td>0,13</td>
</tr>
</tbody>
</table>

A summary of the interlaboratory reproducibility standard deviations ($s_R$) is given in Table 3.

<table>
<thead>
<tr>
<th>(Food) category</th>
<th>(Food) item</th>
<th>$s_R$ values from the interlaboratory study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low inoculation level</td>
</tr>
<tr>
<td>Raw milk and dairy products</td>
<td>Raw milk</td>
<td>0,33</td>
</tr>
<tr>
<td>Raw meat and ready-to-cook meat products (except poultry)</td>
<td>Minced meat</td>
<td>0,24</td>
</tr>
<tr>
<td>Raw poultry and ready-to-cook poultry products</td>
<td>Chicken skin</td>
<td>0,45</td>
</tr>
<tr>
<td>Processed fruits and vegetables</td>
<td>Frozen spinach</td>
<td>0,32</td>
</tr>
<tr>
<td>Primary production samples (PPS)</td>
<td>Broiler caecal material</td>
<td>0,38</td>
</tr>
</tbody>
</table>

12 Test report

[Include 'standard sentences', see main text of this Guidance Document]
Annex A
(normative)

Flow diagram of the procedure

[See example Annex 1 of this Guidance Document]

Annex B
(normative)

Culture media and reagents

B.1 General

[Include ‘standard sentences’, see main text of this Guidance Document]

B.2 Modified charcoal cefoperazone deoxycholate agar (mCCD agar)

B.2.1 Basic medium

B.2.1.1 Composition

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat extract</td>
<td>10,0 g</td>
</tr>
<tr>
<td>Enzymatic digest of animal tissues</td>
<td>10,0 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>(CAS(^2) No. 7647-14-5) 5,0 g</td>
</tr>
<tr>
<td>Activated charcoal</td>
<td>(CAS No. 7440-44-0) 4,0 g</td>
</tr>
<tr>
<td>Enzymatic digest of casein</td>
<td>3,0 g</td>
</tr>
<tr>
<td>Sodium deoxycholate</td>
<td>(CAS No. 302-95-4) 1,0 g</td>
</tr>
<tr>
<td>Iron(II) sulfate hydrate</td>
<td>(CAS No. 13463-43-9) 0,25 g</td>
</tr>
<tr>
<td>Sodium pyruvate</td>
<td>(CAS No. 113-24-6) 0,25 g</td>
</tr>
<tr>
<td>Agar</td>
<td>8,0 g to 18,0 g(^a)</td>
</tr>
<tr>
<td>Water</td>
<td>1 000 ml</td>
</tr>
</tbody>
</table>

\(^a\) Depending on the gel strength of the agar.

B.2.1.2 Preparation

Dissolve the basic components or the dehydrated complete basic medium in the water, by bringing to the boil. Adjust the pH, if necessary, so that after sterilization it is 7,4 ± 0,2 at 20 °C to 25 °C. Dispense the basic medium into flasks of suitable capacity. Sterilize in the autoclave set at 121 °C for 15 min.

\(^2\) CAS Registry Number® is a trademark of CAS corporation. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.
B.2.2 Antibiotic solution

B.2.2.1 Composition

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoperazone sodium salt</td>
<td>0.032 g</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.01 g</td>
</tr>
<tr>
<td>Water</td>
<td>5 ml</td>
</tr>
</tbody>
</table>

B.2.2.2 Preparation

Dissolve the components in the water. Sterilize by filtration.

B.2.3 Complete medium

B.2.3.1 Composition

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic medium (B.3.1)</td>
<td>1000 ml</td>
</tr>
<tr>
<td>Antibiotic solution (B.3.2)</td>
<td>5 ml</td>
</tr>
</tbody>
</table>

B.2.3.2 Preparation

Add the antibiotic solution to the basic medium, cooled down to 44 °C to 47 °C, then mix carefully. Pour 18 ml to 20 ml of the complete medium into sterile Petri dishes (6.6). Allow to solidify. Immediately before use, carefully dry the agar plates, preferably with the lids off and the agar surface downwards, in a drying cabinet for 30 min or until the agar surface is free of visible moisture. If they been prepared in advance, store the undried agar plates in the dark at 5 °C (6.7) for up to one month.
Annex C
(informative)

Performance characteristics of the method

[Introduction text, see example Annex 1 of this Guidance Document]

The values of the performance characteristics, for each (food) item and category, derived from this interlaboratory study are shown in Tables C.1 to C.#, and were calculated in accordance with ISO 17468.

Table C.1— Results of data analysis obtained with broiler caecal material [category: primary production samples (PPS)]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Contamination level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Number of participating collaborators</td>
<td>15</td>
</tr>
<tr>
<td>Number of collaborators retained after evaluation of the data</td>
<td>13</td>
</tr>
<tr>
<td>Number of samples</td>
<td>30</td>
</tr>
<tr>
<td>Number of sample results retained after evaluation of the data</td>
<td>26</td>
</tr>
<tr>
<td>Mean value $\Sigma a$ ($\log_{10}$ cfu/g)</td>
<td>5,1</td>
</tr>
<tr>
<td>Interlaboratory repeatability standard deviation, $s_r$ ($\log_{10}$ cfu/g)</td>
<td>0,13</td>
</tr>
<tr>
<td>Interlaboratory reproducibility standard deviation, $s_R$ ($\log_{10}$ cfu/g)</td>
<td>0,38</td>
</tr>
</tbody>
</table>

NOTE Strain used for inoculation: *C. jejuni* (DSM 24306/CNET 076).

End of Example Annex 2
Annex 3 (informative) Microbiological terms and abbreviated terms

In this annex, a list of microbiological terms and abbreviated terms is given that are used in ISO documents for microbiology of the food chain. This information is summarized to make sure that specific terms and abbreviated terms are described/used in the same way in the different ISO documents. This is a ‘dynamic’ annex allowing the content to grow over time.

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>cfu</td>
<td>colony-forming units (cfu not in capitals)</td>
</tr>
<tr>
<td>LOD\textsubscript{50}</td>
<td>the level of detection for which 50 % of the tests gives a positive result</td>
</tr>
<tr>
<td>log\textsubscript{10} cfu/g</td>
<td>the logarithm to base 10 of the number of cfu per gram</td>
</tr>
<tr>
<td>‘Petri dish’</td>
<td>is written with a capital P as it concerns a ‘dish’ invented by Mr Petri.</td>
</tr>
<tr>
<td>WDCM</td>
<td>World Data Centre for Microorganisms</td>
</tr>
</tbody>
</table>
Guidance Document, 3rd edition

Bibliography


[2] ISO 690, Information and documentation — Guidelines for bibliographic references and citations to information resources


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

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


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

[11] ISO 13307, Microbiology of food and animal feed — Primary production stage — Sampling techniques

[12] ISO 16140 (all parts), Microbiology of the food chain — Method validation

[13] ISO 17468, Microbiology of the food chain — Technical requirements and guidance on establishment or revision of a standardized reference method


[15] ISO 17604, Microbiology of the food chain — Carcass sampling for microbiological analysis

[16] ISO/TS 17728, Microbiology of the food chain — Sampling techniques for microbiological analysis of food and feed samples

[17] ISO 18465:2017, Microbiology of the food chain — Quantitative determination of emetic toxin (cereulide) using LC-MS/MS

[18] ISO 18593, Microbiology of the food chain — Horizontal methods for surface sampling

[19] ISO 19036:2019, Microbiology of the food chain — Estimation of measurement uncertainty for quantitative determinations