
**Microbiology of the food chain —
Technical requirements and guidance
on establishment or revision of a
standardized reference method**

*Microbiologie de la chaîne alimentaire — Exigences et
recommandations techniques pour le développement ou la révision
d'une méthode de référence normalisée*

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Foreword

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

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

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

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

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

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

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Introduction

This International Standard has been developed in order to set common rules for the validation of reference methods standardized in the field of food microbiology by ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, and its corresponding structure at CEN level (CEN/TC 275, *Food analysis — Horizontal methods*, Working Group 6, *Microbiology of the food chain*).

This International Standard refers to the complete process of method validation, including comparison of performance data to predetermined criteria/acceptability values. These values are not set in the present edition of this International Standard, due to the lack of available data.

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Microbiology of the food chain — Technical requirements and guidance on establishment or revision of a standardized reference method

1 Scope

This International Standard gives technical requirements and guidance on the establishment or revision of standardized reference methods for the analysis (detection or quantification) of microorganisms in

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

This International Standard defines the technical stage (or early stage) of the establishment of a new standardized reference method or of the revision of an existing standardized reference method. It includes, in particular, requirements and guidance on the validation of the selected method.

This International Standard is intended to be implemented in particular by ISO/TC 34/SC 9 and its corresponding structure at CEN level, CEN/TC 275, *Food analysis — Horizontal methods*, Working Group 6, *Microbiology of the food chain*.

2 Normative references

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

ISO 16140-1, *Microbiology of the food chain — Method validation — Part 1: Vocabulary*

ISO 16140-2:2016, *Microbiology of the food chain — Method validation — Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 16140-1 and the following apply.

3.1

candidate reference method

method selected and likely to become the *standardized reference method* (3.5)

3.2

multilaboratory study

study of one or several methods conducted in different laboratories, using their own samples analysed in routine

3.3

prestandardization stage

technical stage prior to standardization stage and comprising different steps described in this International Standard

Note 1 to entry: Standardization stage starts with voting on a New Work Item Proposal for inclusion of the Work Item in the work programme.

3.4
“real life” study

study of one or several methods, using a wide range of samples and with preference given to naturally contaminated samples

3.5
standardized reference method

reference method described in a standard

Note 1 to entry: See ISO 16140-1 for the definition of “reference method”.

4 Technical procedure for standardizing a new reference method

4.1 General

In the frame of this International Standard, five technical steps (see 4.2) are required for the validation of a method in view of its standardization as a reference method:

- step 1: method(s) selection;
- step 2: method(s) evaluation study;
- step 3: “real life”/multilaboratory study;
- step 4: selection of the proposed reference method for further validation;
- step 5: interlaboratory study.

Except for step 5 in certain cases (see 4.2.5), these five technical steps correspond to a prestandardization stage (see 3.3), being performed before launching the standardization process.

A flow chart on the technical steps for the establishment of a new standardized reference method is given in Annex A.

4.2 Technical steps

4.2.1 Step 1: Method(s) selection

Information from different sources (national/regional standardized methods, scientific papers on methods with evaluation data, evaluation/validation reports on methods, practicability of the method) shall be made available for the choice of a candidate reference method (see 3.1). Based on the information available, the group in charge of developing the standard selects one or several candidate reference methods.

4.2.2 Step 2: Method(s) evaluation study

An evaluation study of the candidate reference method(s) (see 4.2.1) is conducted, normally by one laboratory, but more than one laboratory may also be involved.

The evaluation of the candidate reference method/each candidate reference method aims at assessing the scope of the method applicability to the range of food categories or environmental sample type to which the method applies. If the method is to be applied to a broad range of foods (general case for standardized reference methods), then at least five categories of food shall be studied (see details in ISO 16140-2:2016, 5.1.3.1). For each food category/sample type, at least one food type and one suitable strain shall be selected. This evaluation study should be conducted in artificial contamination conditions, in order that step 2 and step 3 (see 4.2.3) are complementary to assessing the scope of the method.

This evaluation study should also enable estimation of performance parameters and fulfil the requirements of the method comparison study, as stated in ISO 16140-2:

- for qualitative methods: sensitivity and specificity, level of detection, inclusivity and exclusivity (in accordance with ISO 16140-2:2016, 5.1);
- for quantitative methods: relative trueness (see next paragraph), accuracy profile, inclusivity and exclusivity, and optionally, limit of quantification (in accordance with ISO 16140-2:2016, 6.1).

ISO 16140-2:2016, 5.1 and 6.1 describe a method validation study, comparing an alternative method to a reference method. The methodology described in these subclauses may be used to compare the different candidate reference methods. If one candidate reference method has been selected (see 4.2.1), these subclauses need to be adapted to the case of validating a method alone, without comparison to another method. In particular, reference materials or artificially contaminated samples may be used instead of the reference method to assess the sensitivity and specificity/trueness of the candidate reference method.

NOTE For further details on the procedure for preparing samples, see of ISO 16140-2:2016, Annex C.

If several candidate reference methods have been evaluated at this step, the outcome of this evaluation study should enable the relevant group in charge of developing the standard to reduce the number of candidate reference methods.

4.2.3 Step 3: “Real life”/multilaboratory study

The “real life” study (see 3.4) shall be conducted on the candidate reference method(s) (see 4.2.1), using a wide range of samples with preference given to naturally contaminated samples. This study is a multilaboratory study (see 3.2) conducted in different laboratories, preferably located in different countries/different parts of the world to cover the largest diversity possible of:

- a) matrices where the target microorganism can naturally be found;
- b) strains of the target microorganism.

In particular, each laboratory shall use its own reagents and culture media to reflect their diversity.

If the outcome of this study is not satisfactory, the relevant group in charge of developing the standard shall reconsider the choice of the candidate reference method(s) and come back to step 1.

This “real life” study may be conducted in parallel with step 2 (see 4.2.2).

4.2.4 Step 4: Selection of method for further validation

Based on the information and data gained in the steps 1 to 3 (see 4.2.1 to 4.2.3), the relevant group in charge of developing the standard shall select one method for further validation (see step 5, 4.2.5).

4.2.5 Step 5: Interlaboratory study

An interlaboratory study shall be conducted to adopt a new method selected at step 4 (see 4.2.4). The interlaboratory study is required in particular when it concerns the analysis of a pathogenic microorganism. In exceptional cases, a method may be adopted without an interlaboratory study where it is not possible to conduct one; this decision shall be taken on a case-by-case basis.

The aim of the interlaboratory study is to determine the trueness and precision (repeatability and the reproducibility for quantitative methods) of the selected method implemented in different laboratories using identical samples and to confirm that the method meets the established criteria of performance. Whenever possible, the study conditions should reflect the normal variation between laboratories.

The interlaboratory study should be conducted in accordance with ISO 16140-2:2016, 5.2 for qualitative methods or ISO 16140-2:2016, 6.2 for quantitative methods. The interlaboratory study should include one matrix per food category studied in step 2 (see 4.2.2).