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Microbiology of the food chain — Method validation —

Part 4: Protocol for method validation in a single laboratory

Microbiologie de la chaîne alimentaire — Validation des méthodes —

Partie 4: Protocole pour la validation de méthodes dans un seul laboratoire

Reference number
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Foreword

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

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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).

A list of all parts in the ISO 16140 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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Introduction

0.1 The ISO 16140 series

The ISO 16140 series has been expanded in response to the need for various ways to validate or verify test methods. It is the successor to ISO 16140:2003. The ISO 16140 series consists of six parts with the general title, *Microbiology of the food chain — Method validation:*

— *Part 1: Vocabulary*;

— *Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method*;

— *Part 3: Protocol for the verification of reference methods and validated alternative methods in a single laboratory*;

— *Part 4: Protocol for method validation in a single laboratory*;

— *Part 5: Protocol for factorial interlaboratory validation for non-proprietary methods*;

— *Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures*.

ISO 17468 is a closely linked International Standard, which establishes technical rules for the development and validation of standardized methods.

In general, two stages are needed before a method can be used in a laboratory.

— The first stage is the validation of the method. Validation is conducted using a study in a single laboratory followed by an interlaboratory study (see ISO 16140-2, ISO 16140-5 and ISO 16140-6). In the case when a method is validated within one laboratory (as described in this document), no interlaboratory study is conducted.

— The second stage is method verification, where a laboratory demonstrates that it can satisfactorily perform a validated method. This is described in ISO 16140-3. Verification is only applicable to methods that have been validated using an interlaboratory study.

In general, two types of methods are distinguished: reference methods and alternative methods.

A reference method is defined in ISO 16140-1:2016, 2.59, as an "internationally recognized and widely accepted method". The note to entry clarifies that "these are ISO standards and standards jointly published by ISO and CEN or other regional/national standards of equivalent standing".

In the ISO 16140 series, reference methods include standardized reference (ISO and CEN) methods as defined in ISO 17468:2016, 3.5, as a "reference method described in a standard".

An alternative method (method submitted for validation) is defined in ISO 16140-1:2016, 2.4, as a "method of analysis that detects or quantifies, for a given category of products, the same analyte as is detected or quantified using the corresponding reference method". The note to entry clarifies that: "The method can be proprietary. The term 'alternative' is used to refer to the entire 'test procedure and reaction system'. This term includes all ingredients, whether material or otherwise, required for implementing the method.".

This document, ISO 16140-4, addresses validation within a single laboratory. The results are therefore only valid for the laboratory that conducted the study. In this case, verification (as described in ISO 16140-3) is not applicable. ISO 16140-5 describes protocols for non-proprietary methods where a more rapid validation is required or when the method to be validated is highly specialized and the number of participating laboratories required by ISO 16140-2 cannot be reached. This document and ISO 16140-5 can be used for validation against a reference method. This document (regarding qualitative and quantitative methods) and ISO 16140-5 (regarding quantitative methods only) can also be used for validation without a reference method.

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The flow chart in Figure 1 gives an overview of the links between the different parts mentioned above. It also guides the user in selecting the right part of the ISO 16140 series, taking into account the purpose of the study and the remarks given above.

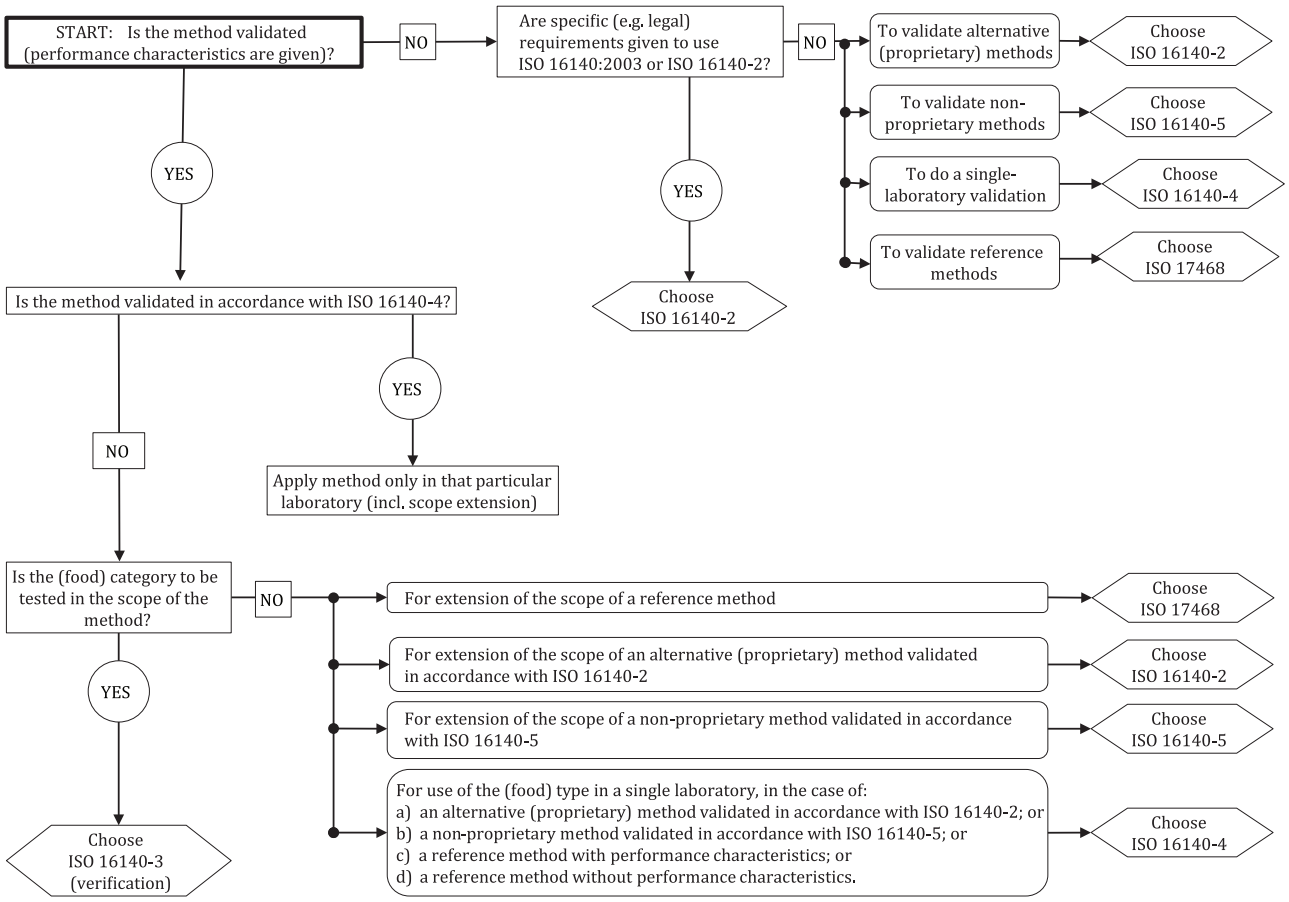

Figure 1 — Flow chart for application of the ISO 16140 series

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.

ISO 16140-6 is somewhat different from the other parts in the ISO 16140 series in that it relates to a very specific situation where only the confirmation procedure of a method is to be validated [e.g. the biochemical confirmation of *Enterobacteriaceae* (see ISO 21528-2)]. The confirmation procedure advances a suspected (presumptive) result to a confirmed positive result. The validation of alternative typing techniques (e.g. serotyping of *Salmonella*) is also covered by ISO 16140-6. The validation study in ISO 16140-6 clearly defines the selective agar(s) from which strains can be confirmed using the alternative confirmation method. If successfully validated, the alternative confirmation method can only be used if strains are recovered on an agar that was used and shown to be acceptable within the validation study. Figure 2 shows the possibilities where an alternative confirmation method validated in accordance with ISO 16140-6 can be applied (see text in the boxes).

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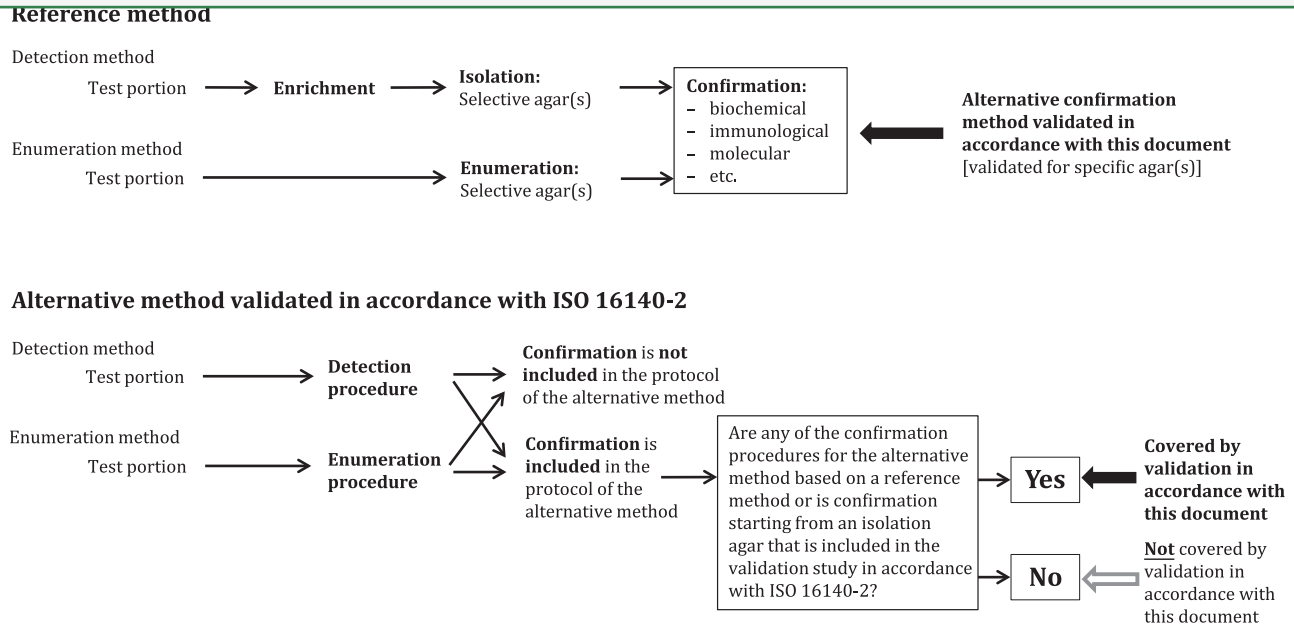

Figure 2 — Use of validated alternative confirmation methods (see ISO 16140-6)

EXAMPLE An example application of a validated alternative confirmation method is as follows.

An alternative confirmation method based on ELISA has been validated (in accordance with ISO 16140-6) to replace the biochemical confirmation for *Salmonella* as described in ISO 6579-1. In the validation study, XLD (mandatory agar in accordance with ISO 6579-1) plus BGA and a specified chromogenic agar (two optional agars for second plating in accordance with ISO 6579-1) were used as the agars to start the confirmation. The validated confirmation method can be used to replace the biochemical confirmation under the following conditions:

— by laboratories using the ISO 6579-1; or

— by laboratories using an ISO 16140-2 validated alternative method that refers to ISO 6579-1 for confirmation; or

— by laboratories using an ISO 16140-2 validated alternative method that starts the confirmation from XLD and/or BGA agar and/or the specified chromogenic agar.

The validated confirmation method cannot be used under the following conditions:

— by laboratories using an ISO 16140-2 validated alternative method that refers only to agars other than those included in the validation to start the confirmation (e.g. Hektoen agar and SS agar only); or

— by laboratories using an ISO 16140-2 validated alternative method that refers only to a confirmation procedure that does not require isolation on agar.

0.2 Validation protocols in the ISO 16140 series

An interlaboratory validation study, in accordance with ISO 16140-2, requires at least eight laboratories for quantitative methods and at least ten laboratories for qualitative methods. ISO 16140-5 is intended to be used for interlaboratory studies comprising four to seven laboratories for quantitative methods and four to nine laboratories for qualitative methods. ISO 16140-5 can only be used for non-proprietary methods. Table 1 provides an overview of the different protocols.

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Table 1 — Overview of different validation protocols described in the ISO 16140 series

Number of participating laboratories	With reference method	Without reference method
1	This document: — factorial (see 5.1.1 and 5.2.1), or — conventional (see 6.1.1 and 6.2.1)	This document: — factorial (see 5.1.2 and 5.2.2), or — conventional (see 6.1.2 and 6.2.2)
4 to 7 (quantitative method)/ 4 to 9 (qualitative method)	ISO 16140-5: for non-proprietary methods only	ISO 16140-5: for non-proprietary quantitative methods only
≥ 8 (quantitative method)/ ≥ 10 (qualitative method)	ISO 16140-2: for the interlaboratory study part	Not applicable

The aim of this document is to assess the performance of detection or quantification methods within a single laboratory, typically across a number of (food) categories and (food) types. Single-laboratory validation of alternative methods for microbiological confirmation and typing procedures can also be performed under certain conditions: the general principles are the same as those described in ISO 16140-6 for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures (except there is no interlaboratory study). Further information is given in Annex G.

The protocols in this document only validate the method for the particular laboratory. A generalization to other laboratories is not within the scope of these protocols. However, extension to other laboratories is possible if this document is used as the first phase of validation of a reference method, to be followed by an interlaboratory study as described in ISO 17468.

If a reference method is available, the validation of a method is conducted by comparing the alternative method to the reference method. This allows inclusion of naturally contaminated samples in the validation process and thus provides a more realistic picture of the performance of the method. If no reference method is available, the validation process is based on samples with known contamination levels only. This document provides protocols for both situations.

The general principles for single-laboratory validations of detection and quantification methods are the same as those described in ISO 16140-2 for the validation of alternative (proprietary) methods against a reference method. This document cannot be used without ISO 16140-1 or ISO 16140-2, as many definitions and procedures are given in these International Standards. In addition to the validation parameters described in ISO 16140-2, this document describes the calculation of in-house repeatability and in-house reproducibility. Calculation of these parameters is not required if an interlaboratory study is to be conducted after the single-laboratory validation (i.e. if the single-laboratory validation is only the first phase of validation). Reliability of performance parameters obtained with this document is comparable to ISO 16140-2. This also means that the workload associated with the technical protocols for the single laboratory is comparable with the method comparison study of ISO 16140-2.

This document provides two strategies for the single-laboratory method validation of detection and quantification methods. The first strategy is based on a factorial approach while the second strategy uses the conventional approach derived from the protocols of ISO 16140-2. In addition, protocols for the determination of the in-house reproducibility for quantitative methods are described.

The advantages of using a factorial approach, over the conventional approach, are that it takes into account specific conditions that the laboratory encounters during routine testing and provides more information on the factors (technicians, culture media, etc.) that vary within the laboratory across relevant (food) items, while using fewer samples to assess the performance of the method. The factorial approach offers assessment of the precision of quantitative methods. It allows computation of reliable and representative single-laboratory method validation parameters such as in-house reproducibility standard deviation, LOD_{50} or RLOD values because it provides information on the variability of these values under different measurement conditions. The factorial approach requires fewer test results in order to obtain similar or higher levels of reliability compared to the conventional approach.

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