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Microbiology of the food chain — Method validation —

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

Microbiologie de la chaîne alimentaire — Validation des méthodes — Partie 6: Protocole pour la validation de méthodes alternatives (commerciales) pour la confirmation microbiologique et le typage

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Contents

Page

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).

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 of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

A list of all parts of 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.

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 validated 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 as described in this document). In the case when a method is validated within one laboratory (see ISO 16140-4), 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.”.

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. ISO 16140-4 and ISO 16140-5 can be used for validation against a reference method. ISO 16140-4 (qualitative and quantitative) and ISO 16140-5 (quantitative only) can also be used for validation without a reference method.

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.

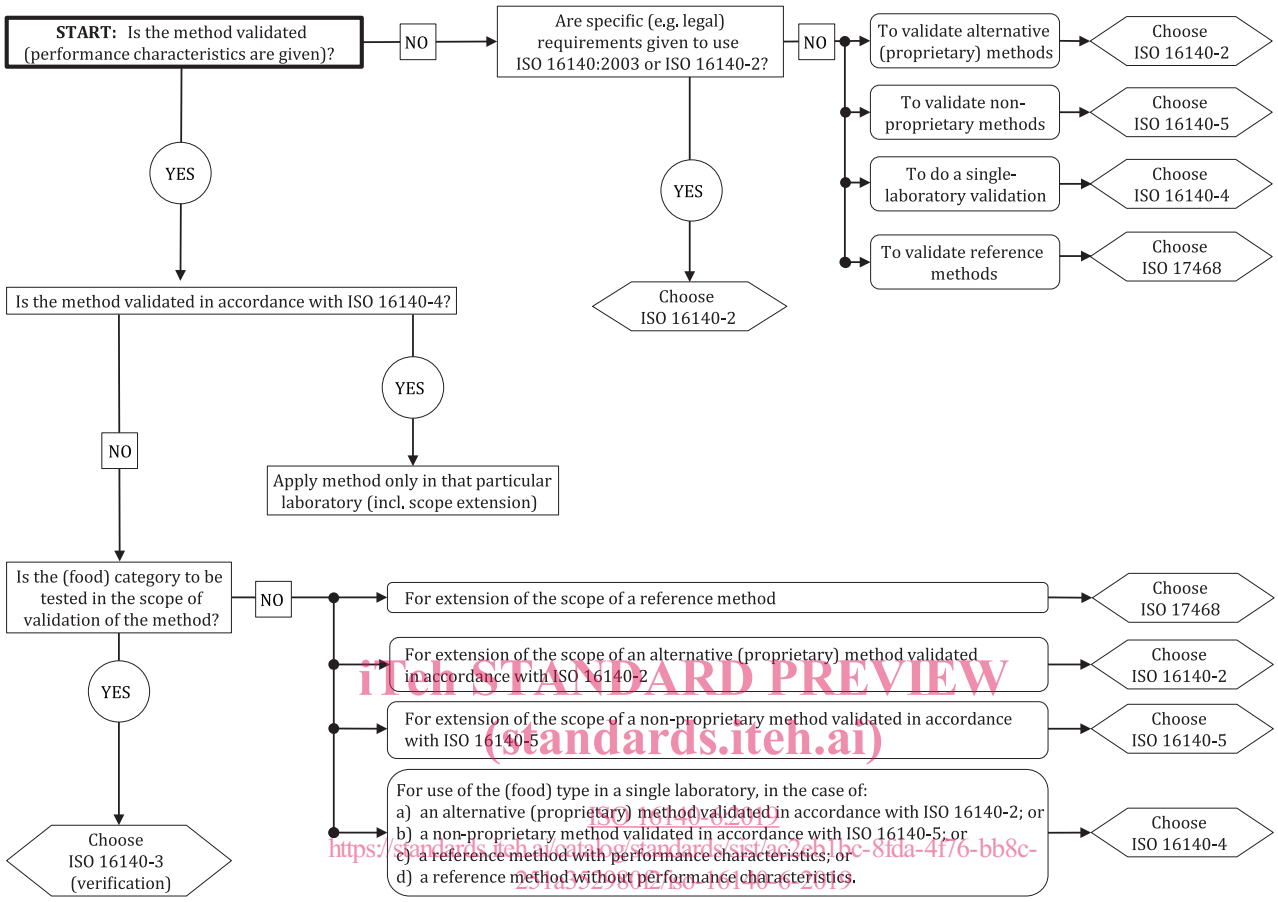

Figure 1 — Flow chart for application of the ISO 16140 series

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.

This document, 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 this document. The validation study in this document 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 this document can be applied (see text in the boxes).

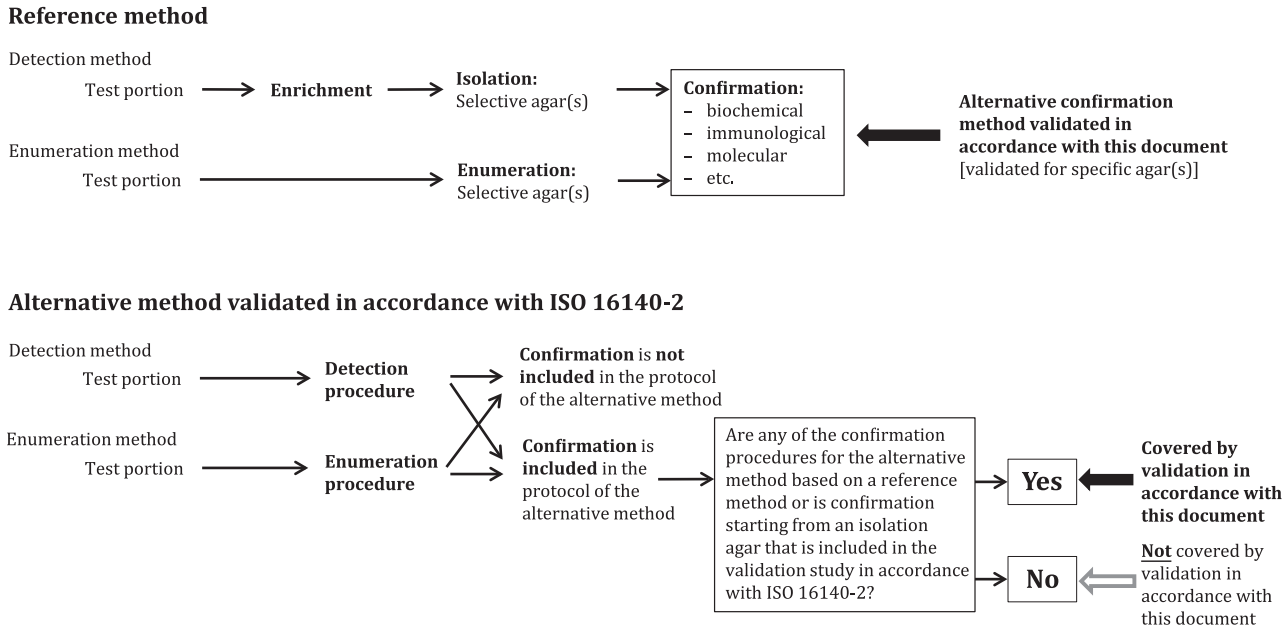

Figure 2 — Use of validated alternative confirmation methods (described in this document)

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

An alternative confirmation method based on ELISA has been validated 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 and verification of methods for the microbiological confirmation and typing procedures

The procedure described in this document is intended for the "full" validation of alternative (proprietary) methods for microbiological confirmation and/or typing, hereafter referred to as "alternative confirmation methods".

During the validation study, the performance of the alternative confirmation method is compared to the performance of the reference confirmation procedure.

The procedure for verification of alternative confirmation methods in a single laboratory is described in ISO 16140-3.

Microbiology of the food chain — Method validation —

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

1 Scope

This document specifies the general principle and the technical protocol for the validation of alternative confirmation methods for microbiology in the food chain. This document compares the result of the alternative confirmation method against the confirmation procedure of a reference method or, if needed, a reference confirmation method (e.g. whole genome sequencing).

This document is applicable to the validation of alternative confirmation methods used for the analysis (detection or quantification) of isolated microorganisms in:

— products intended for human consumption;

— products intended for animal feeding;

— environmental samples in the area of food and feed production, handling;

— samples from the primary production stage.

Validated alternative confirmation methods can be used to replace (partly or completely) the confirmation procedure described in:

— the reference method;

— an alternative method validated in accordance with ISO 16140-2 only if one of the isolation agars specified in the validation study of the alternative confirmation method is used.

This document is also applicable to the validation of alternative typing methods, where the reference method can be, for example, a serological method (e.g. serotyping of *Salmonella*) or a molecular method (e.g. typing of Shiga toxin-producing *E. coli*).

This document is, in particular, applicable to bacteria and fungi. Some clauses can be applicable to other (micro)organisms, to be determined on a case-by-case basis.

Validation studies in accordance with this document are primarily intended to be performed by organizations or expert laboratories involved in method validation, but can also be used by a single laboratory, especially when performing in-house validation under certain conditions (see ISO 16140-4).

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 16140-1:2016, *Microbiology of the food chain — Method validation — Part 1: Vocabulary*

3 Terms and definitions

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

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

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

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

3.1
acceptability limit
AL
maximum positive or negative acceptable difference between the reference value (or if not known, the accepted reference value) of a sample and an individual result obtained when applying the operating procedure of an analytical method

Note 1 to entry: Annex D provides further information on the use of AL for this document.

[SOURCE: ISO 16140-1:2016, 2.1, modified — Note 1 to entry has been replaced.]

3.2
alternative confirmation or typing method
confirmation or typing method submitted for validation
method of analysis that confirms or types the same analyte as is confirmed or typed using the corresponding reference method

Note 1 to entry: 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.

Note 2 to entry: For clarity of reading, the text in this document generally describes validation of a confirmation method (detailed examples are given in Annex B). If applicable, this can be read as validation of a typing method (detailed examples are given in Annex C).

3.3
confirmation procedure
number of defined *confirmation tests* (3.4) that are performed on a strain, the combined results of which are used to definitively confirm the identity of that strain

3.4
confirmation test
single test which is carried out to verify a presumptive result

Note 1 to entry: The result of a single test may not on its own be able to definitively confirm the identity of the strain.

[SOURCE: ISO 16140-1:2016, 2.17, modified — In the term and definition, "procedure or" has been removed, "single" has been added and the Note 1 to entry has been replaced.]

3.5
microbial (sub)type
group of closely related microorganisms (within a species) distinguished by their shared specific characteristics as determined by, for example, serological testing (serotype) or molecular testing (genotype)

3.6
non-target strain
strain, defined according to the scope of the reference method, that would not reasonably be expected to be confirmed by the alternative method

[SOURCE: ISO 16140-1:2016, 2.44, modified — In the definition, "confirmed" has replaced "detected or enumerated".]

3.7
reference confirmation or typing procedure
combination of the confirmation or typing tests that are claimed to be replaced by the *alternative confirmation or typing method* (3.2)

Note 1 to entry: The number of *confirmation tests* (3.4) depends on the reference method for the specific microorganisms. The number of confirmation tests can also be one.

3.8
target strain
strain, defined according to the scope of the reference method, that is expected to be confirmed by the alternative method

[SOURCE: ISO 16140-1:2016, 2.74, modified — In the definition, "confirmed" has replaced "detected or enumerated".]

3.9
typing procedure
process of determining a particular *microbial (sub)type* (3.5)

4 General principles for the validation of confirmation and typing methods

In the validation study, the alternative confirmation method is compared to the confirmation procedure described in the reference method for the enumeration or detection of specific (groups of) microorganisms.

The validation protocol comprises two phases:

— a method comparison study, of the alternative confirmation method against the reference confirmation procedure, carried out in the organizing laboratory;

— an interlaboratory study.

NOTE It is possible, if relevant, to include inclusivity or exclusivity data obtained in an ISO 16140-2 validation study into a study related to this document.

The validation protocol shall clearly define the selective agar(s) from which strains can be confirmed using the alternative confirmation method. All inclusivity and exclusivity strains shall be tested. In cases where some strains are unable to grow on the specified selective agar(s), a non-selective agar plate shall also be included in the method comparison study and the interlaboratory study.

The technical rules for performing the method comparison study and the interlaboratory study are given in Clauses 6 and 7. The following six cases are covered; a distinction is made between the confirmation/typing of *Salmonella* and that of other microorganisms:

— validation of methods used for confirmation to the family level (non-*Salmonella*);

— validation of methods used for confirmation to the genus level (non-*Salmonella*);

— validation of methods used for confirmation to the species level (non-*Salmonella*);

— validation of methods used for confirmation/typing to the microbial (sub)type level (non-*Salmonella*);

— validation of methods used for confirmation/typing to the *Salmonella* genus or species level;

— validation of methods used for confirmation/typing to the *Salmonella* serovar level.

5 Strains

The pure strains used for determining the inclusivity and the exclusivity shall be well-characterized in line with the purpose of the validation study. The identification information of each strain will be used to (additionally) confirm the result in cases of discrepancies between the results of the reference confirmation procedure and the alternative confirmation method.

NOTE National, regional or international reference laboratories could be contacted during such investigations.

6 Method comparison study

6.1 General

The method comparison study is the part of the validation that is performed in one laboratory. It consists of an inclusivity and exclusivity study of the alternative confirmation method. The results are then compared to those of the reference confirmation procedure.

6.2 Selection of test strains

A range of strains shall be used. Criteria for selecting test strains are given in Annex A. The strains selected should take into account the measurement principle (e.g. culture-based, immunological, molecular-based) of the alternative method. Different measurement principles may require the use of panels of different test strains, representing the diversity of the studied microorganism(s). It is important to include non-target microorganisms that may grow on the media used for the reference and for the alternative method, including those that produce suspect colonies (i.e. look like those produced by the target strains).

The rationale for the choice of the strains and their characteristics shall be included in the validation study report.

Each strain shall be characterized biochemically and/or serologically and/or genetically in sufficient detail for its identity to be known. Strains should preferably have been isolated from foods, feed, the food-processing environment or from primary production; depending on the scope of the validation. However, clinical, environmental and culture collection strains can also be used. The original source of all strains should be known, and they should be held in a local (e.g. expert laboratory), national or international culture collection to enable them to be used in future testing if required. See ISO 11133 for guidance on the local maintenance of stock cultures.

Results generated by a specialized reference laboratory, using the reference method, can be used if the laboratory performing the validation study is not able to perform the confirmation/typing of rare strains according to the reference method. For example, the use of serotyping results of a *Salmonella* reference laboratory is allowed in cases of rare *Salmonella* serovars.

6.3 Inclusivity study

6.3.1 Testing of target strains

Pure cultures of all target strains shall be tested with both the reference confirmation procedure and the alternative confirmation method. It is not necessary to repeat the reference confirmation procedure along with the alternative confirmation method if the required data for the reference procedure are available. As all inclusivity strains shall be tested, subculture the strains on a non-selective agar plate, along with the clearly defined selective agar(s) from which strains can be confirmed using the alternative confirmation method. This will ensure that a viable strain is available for confirmation.

The number of strains to be tested under the various options (see 6.3.2 to 6.3.7) are summarized in Tables D.1 and D.2.

