SLOVENSKI STANDARD
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Mikrobiologija v prehranski verigi - Validacija metode - 6. del: Protokol za validacijo alternativnih (lastniških) metod za postopke mikrobiološke potrditve in tipizacije (ISO/DIS 16140-6:2017)

Microbiology of the food chain - Method validation - Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures (ISO/DIS 16140-6:2017)

Mikrobiologie von Lebensmitteln und Futtermitteln - Verfahrensvalidierung - Teil 6: Arbeitsvorschrift für die Validierung mikrobiologischer Nachweisverfahren (ISO/DIS 16140-6)

Microbiologie de la chaîne alimentaire - Validation des méthodes - Partie 6: Protocole pour la validation des méthodes alternatives (propriétaires) pour confirmation et identification microbiologique (ISO/DIS 16140-6:2017)

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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 des méthodes alternatives (propriétaires) pour confirmation et identification microbiologique

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ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

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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).

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For an explanation on 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 the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products,* Subcommittee SC 9, *Microbiology* (Working Group WG 3, *Method validation).*

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

Introduction

The ISO 16140 series has been elaborated in response to the need for various ways to validate or verify test methods. It is the successor of ISO 16140:2003, *Microbiology of food and animal feeding stuffs — Protocol for the validation of alternative methods.* ISO 16140 series consists of several 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 and validated alternative methods implemented in a single laboratory*

— *Part 4: Protocol for single-laboratory (in-house) method validation*

— *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, *Microbiology of the food chain — Technical requirements and guidance on establishment or revision of a standardized reference method*[7], is a closely linked International Standard. This International Standard, which establishes technical rules for the development and validation of standardized methods, is intended for the development of standardized methods by ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology* and CEN/TC 275/WG 6, *Microbiology of the food chain.*

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

— The first stage is the validation of the method. This is either conducted in several laboratories (parts 2 and 5 of ISO 16140) or in one laboratory (part 4 of ISO 16140).

— The second stage is method verification, where a laboratory demonstrates that it can satisfactorily perform a validated method. This is described in part 3 of ISO 16140 (method verification). In part 3, a separation is made between verification of (food) items that are included in the validation study and (food) items that are not tested in the validation study but belong within the scope of validation.

NOTE 1 Standardized reference methods (with and without published validation data) only require verification before implementation in the laboratory.

NOTE 2 In this part of ISO 16140, the word 'item' is sometimes combined with 'food' to improve the readability of this document. However, the word 'food' is interchangeable with 'feed' and the other areas of the food chain as mentioned in the Scope of ISO 16140-6.

Part 4 of ISO 16140 addresses validation within a single laboratory. The results are therefore only valid in the laboratory which conducted the study. In this case, verification (part 3 of ISO 16140) is not required.

Part 5 of ISO 16140 describes protocols for situations where a more rapid validation is required or when the method to be validated is highly specialised, and, the number of participating laboratories required by ISO 16140-2 cannot be reached.

The flow chart in Figure 1 gives an overview of the links between the different parts mentioned above. It also guides the users in selecting the right part of the ISO 16140 series, taking into account the purpose of the study and the remarks given above. For this, it is important to distinguish between 'reference method' and 'standardized reference method'. A reference method is an internationally recognized and widely accepted method (term 2.59 of ISO 16140-1:2016) and a standardized reference method is a reference method described in a standard (term 3.5 of ISO 17468:2016). In the ISO 16140 series, reference method includes standardized reference method. The flow diagram acknowledges that published validation data may not be available for some standardized reference methods.

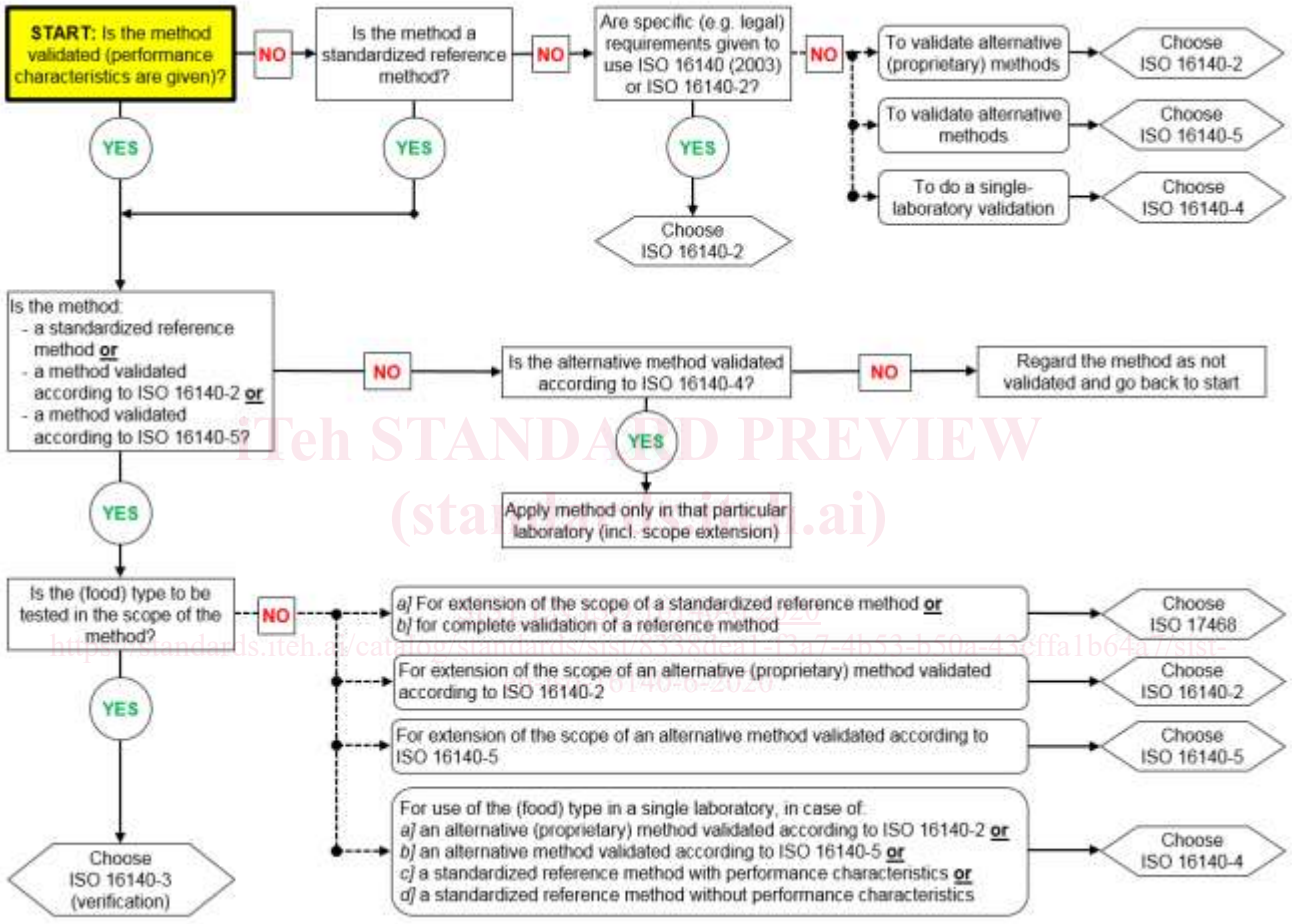

Figure 1 — Flow chart for application of the different parts of the ISO 16140-series

Part 6 of ISO 16140, 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 validated. The confirmation procedure advances a suspected (presumptive) result to a confirmed positive result. The typing of pure strains (e.g. serotyping of *Salmonella*) is included in part 6 of ISO 16140.

The procedure described in this part of ISO 16140 is intended for the 'full' validation of alternative (proprietary) methods for microbiological confirmation and typing, further referred to as 'alternative confirmation methods'. The procedure for verification of alternative confirmation methods in a single laboratory will be described in part 3 of ISO 16140.

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

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 part of ISO 16140 specifies the general principle and the technical protocol for the validation of alternative, mostly proprietary, confirmation methods for microbiology in the food chain. This part of ISO 16140 compares the result of the alternative confirmation method against that of the reference confirmation procedure.

This part of ISO 16140 is applicable to the validation of alternative confirmation methods used in the analysis (detection or quantification) of microorganisms in

— products intended for human consumption,

— products intended for animal feeding,

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

— 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; and

— an alternative method validated according to part 2 of ISO 16140 [5] as long as one of the isolation agars specified in the validation study of the alternative confirmation method is used.

This part of ISO 16140 is also applicable to the validation of alternative typing methods, where the reference method for example can be a serological method (e.g. serotyping of *Salmonella*) or a molecular method (e.g. typing of Shiga toxin-producing *E. coli*).

This part of ISO 16140 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 according to this part of ISO 16140 are intended to be performed by organizations involved in method validation and not by single user laboratories.

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

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:

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

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

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

[SOURCE: ISO 16140-1:2016, 2.1, modified]

Note 1 to entry: Annex D provides further information on the use of acceptability limit (AL) for this part of ISO 16140.

3.2
confirmation procedure
number of defined tests that are performed on a strain, the combined results of which are used to definitively confirm the identity of that strain

3.3
confirmation test
single test which is carried out to verify a presumptive result

[SOURCE: ISO 16140-1:2016, 2.17, modified]

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

3.4
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]

3.5
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

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

Note 2 to entry: A detailed example is given in Annex B and in Annex C.

Note 3 to entry: For clarity of reading the text of this document is generally described for validation of a confirmation procedure (Annex B). If applicable this can be read as typing procedure (Annex C).

3.6
microbial (sub)type
a group of closely related microorganisms (within a species) distinguished by their shared specific characteristics as determined by e.g. serological testing (serotype) or molecular testing (genotype)

3.7
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]

3.8
typing
the process of determining a particular microbial (sub)type

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 (Clause 6);

— an interlaboratory study (Clause 7).

The validation protocol shall clearly define the selective agars 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 according to part 6 of ISO 16140 can be applied (see text in **red**).

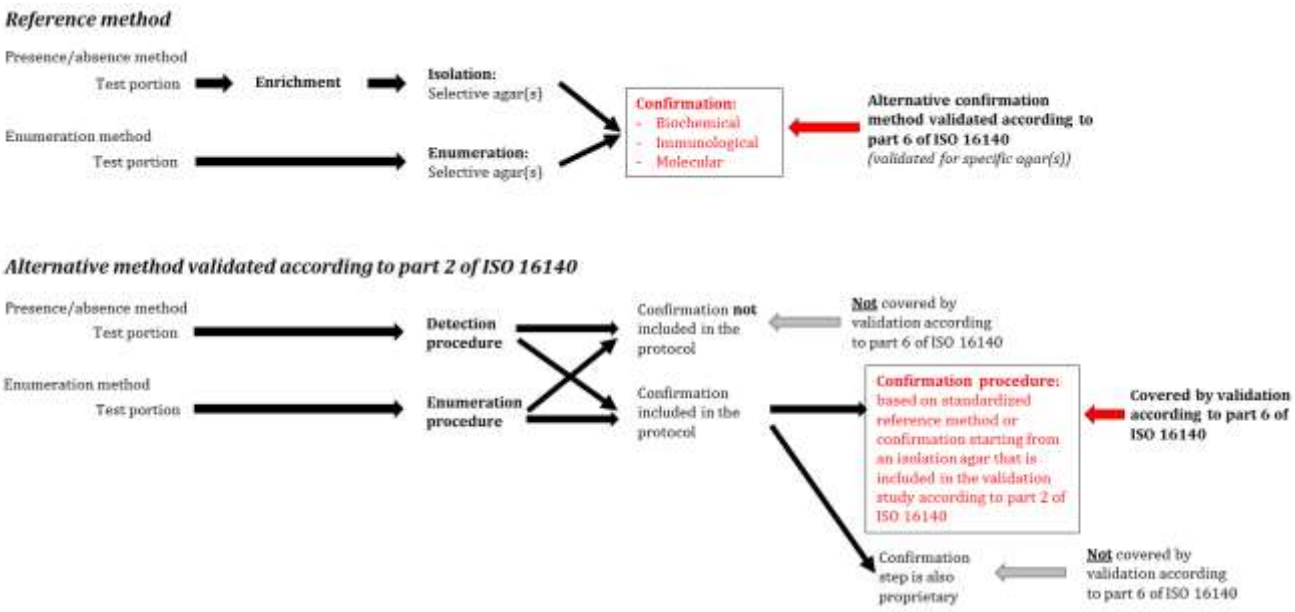

Figure 2 — Use of alternative confirmation methods validated according to part 6 of ISO 16140
(see text in red)

EXAMPLE Application of a validated alternative confirmation method:

An alternative confirmation method based on PCR is validated to replace the biochemical confirmation for *Salmonella* as described in ISO 6579-1[1]. In the validation study XLD (mandatory agar according to ISO 6579-1) and BGA (optional agar according to ISO 6579-1) were used as the agars to start the confirmation. The validated confirmation method can be used under the following conditions:

1) by laboratories using the ISO 6579-1 to replace the biochemical confirmation, or

2) by laboratories using an ISO 16140-2 validated alternative method that refers to ISO 6579-1 for (biochemical) confirmation, or

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

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

1) by laboratories using an ISO 16140-2 validated alternative method that refers to a proprietary method for confirmation (e.g. a chromogenic agar), or

2) by laboratories using an ISO 16140-2 validated alternative method that refers to other agars to start the confirmation (e.g. Hektoen agar), or

3) by laboratories using an ISO 16140-2 validated alternative method that refers to a confirmation procedure not based on isolation on agar (e.g. an ELISA test).

NOTE It is possible to include data already obtained in an inclusivity or exclusivity part of an ISO 16140-2[6] study into an ISO 16140-6 study, if relevant.

The technical rules for performing the method comparison study and the interlaboratory study are given in Clause 6 and Clause 7. The following 6 cases will be 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 have to be well characterised: therefore, strain characterisation shall follow the requirements as specified in ISO 7218. This 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.

