SLOVENSKI STANDARD
oSIST prEN ISO 16140-7:2023

01-julij-2023

Mikrobiologija v prehranski verigi - Validacija metode - 7. del: Protokol za validacijo metod za identifikacijo mikroorganizmov (ISO/DIS 16140-7:2023)

Microbiology of the food chain - Method validation - Part 7: Protocol for the validation of identification methods of microorganisms (ISO/DIS 16140-7:2023)

Mikrobiologie der Lebensmittelkette - Verfahrensvalidierung - Teil 7: Arbeitsvorschrift für die Validierung von Identifizierungsverfahren von Mikroorganismen (ISO/DIS 16140 7:2023)

Microbiologie de la chaîne alimentaire - Validation des méthodes - Partie 7: Protocole pour la validation de méthodes d'identification des micro-organismes (ISO/DIS 16140-7:2023)

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Microbiology of the food chain — Method validation —

Part 7:
Protocol for the validation of identification methods of microorganisms

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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 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*, 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 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 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 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*;

— *Part 7: Protocol for the validation of identification methods of microorganisms.*

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, ISO 16140-6 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/DIS 17468, 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 (regarding qualitative and quantitative methods)

and ISO 16140-5 (regarding quantitative methods 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.

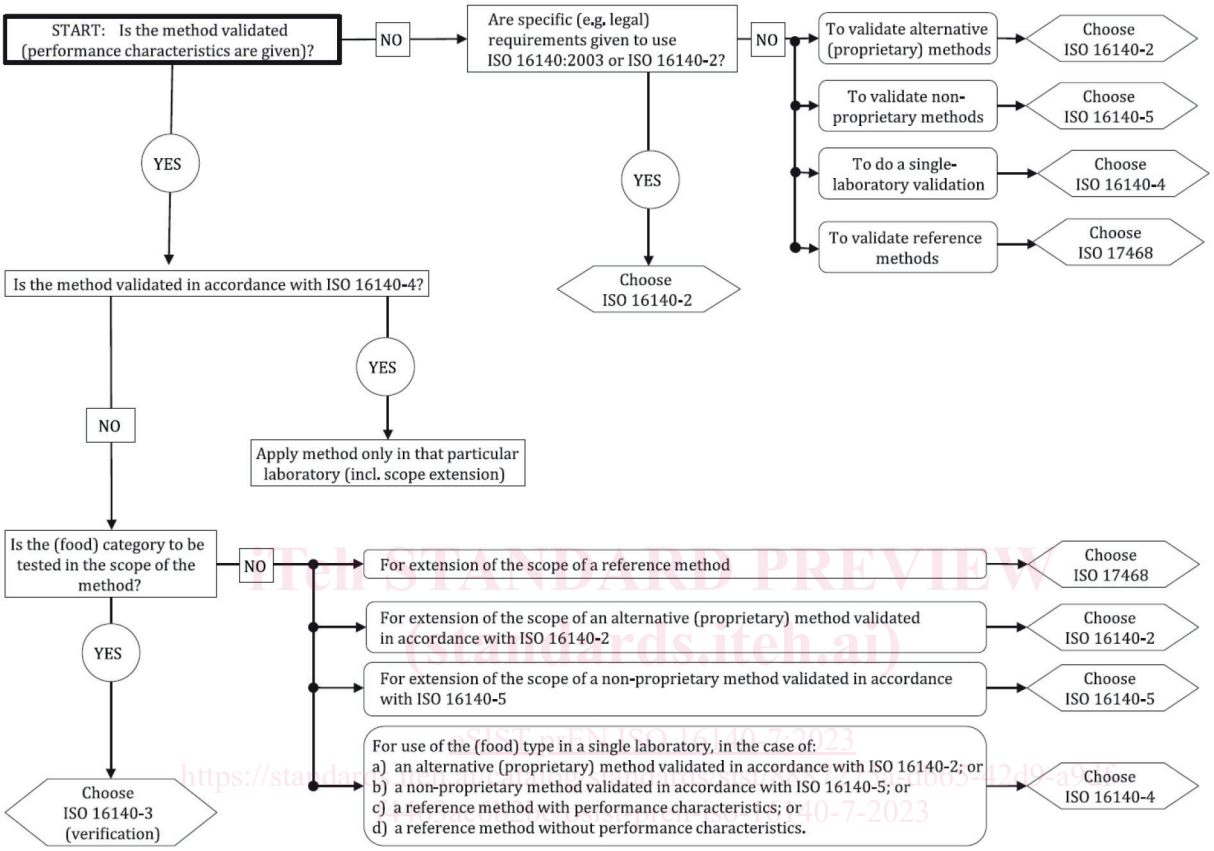
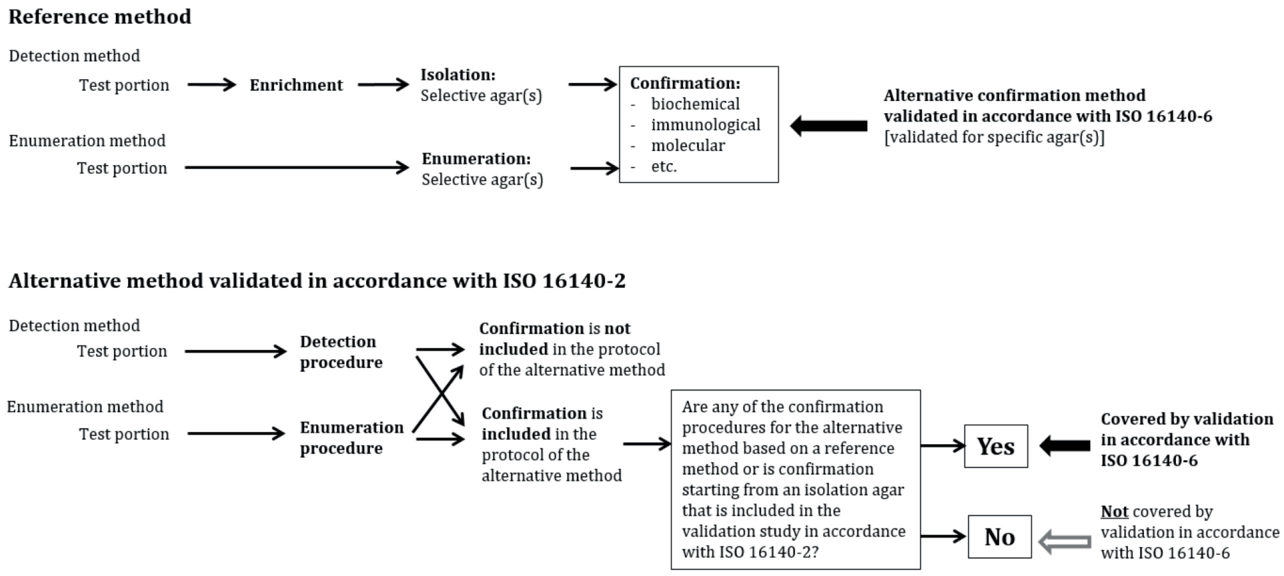


Figure 1 — Flow chart for application of the ISO 16140 series (parts 2, 3, 4 and 5)

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 and ISO 16140-7 are somewhat different from the other parts in the ISO 16140 series in that they relate to very specific situations.

ISO 16140-6 is restricted to the confirmation procedure of a method 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 was 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).



Reference method
Detection method
Test portion
Enrichment
Isolation:
Selective agar(s)
Confirmation:
- biochemical
- immunological
- molecular
- etc.
Alternative confirmation method validated in accordance with ISO 16140-6
[validated for specific agar(s)]
Enumeration method
Test portion
Enumeration:
Selective agar(s)
Alternative method validated in accordance with ISO 16140-2
Detection method
Test portion
Detection procedure
Confirmation is not included in the protocol of the alternative method
Enumeration method
Test portion
Enumeration procedure
Confirmation is included in the protocol of the alternative method
Are any of the confirmation procedures for the alternative method based on a reference method or is confirmation starting from an isolation agar that is included in the validation study in accordance with ISO 16140-2?
Yes
Covered by validation in accordance with ISO 16140-6
No
Not covered by validation in accordance with ISO 16140-6

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

This document, ISO 16140-7, addresses the validation of identification procedures (e.g. molecular identification using multiplex PCR or DNA sequencing or mass spectrometry). This document differs from the other parts in the ISO 16140 series, as there is no reference method for microbial identification and, therefore, it is not possible to run a method comparison study. The validation study in this document defines an identification database and agar(s) from which strains can be identified. If properly characterized and successfully validated, the identification method can only be validly used on strains recovered on the agars covered and shown to have been acceptable within the validation study.

Figure 3 shows the possibilities where an alternative confirmation method validated in accordance with ISO 16140-6 and an alternative identification method validated in accordance with ISO 16140-7 can be applied within a reference method or an ISO 16140-2 validated detection or enumeration method. The result provided by the ISO 16140-7 validated method can be considered as additional information on the identity of the tested colony(ies); this result cannot be taken as a confirmation result. When there is a discrepancy between the results of the ISO 16140-6 validated method and the ISO 16140-7 validated method, a root cause analysis is conducted. An ISO 16140-7 validated method can also be used to identify colonies within methods that do not require a confirmation step.

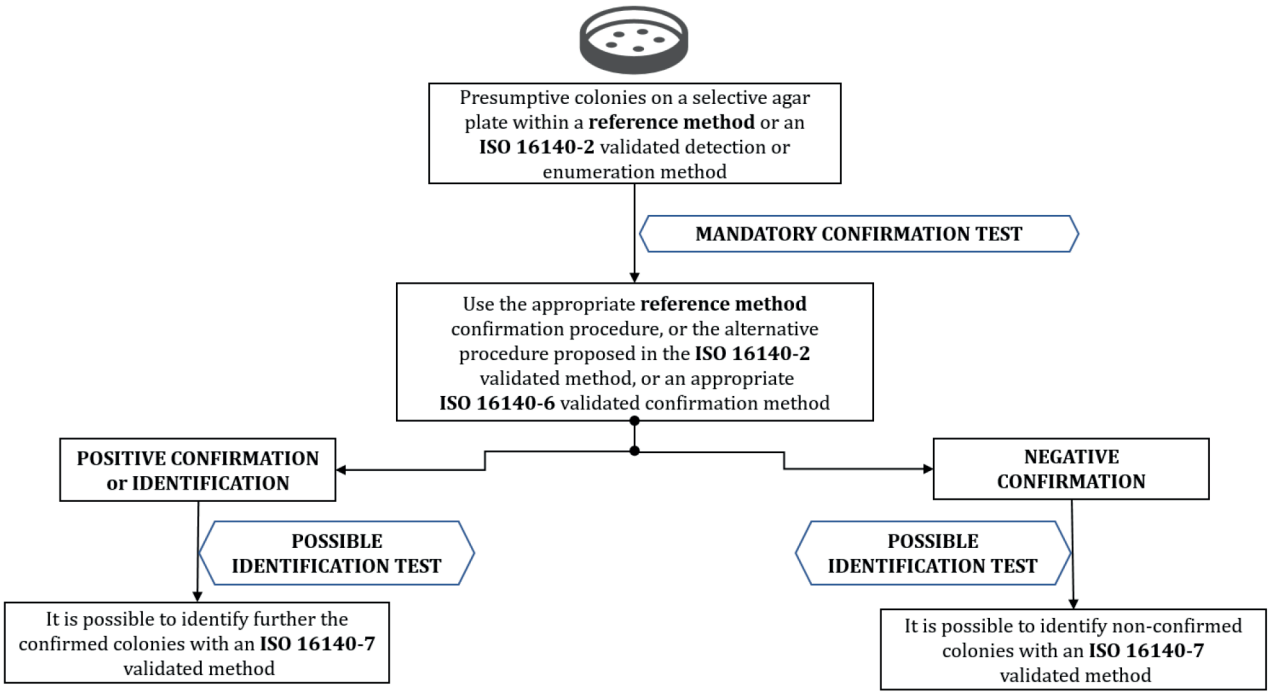


Figure 3 — Flow chart for the application of ISO 16140-6 and ISO 16140-7 for the confirmation and identification of colonies within a reference method or an ISO 16140-2 validated detection or enumeration method

If the identification method is as well validated in accordance with ISO 16140-6, the same method can be used for both, confirmation and identification.

When a confirmation method is used, it is possible to apply an identification method validated in accordance with ISO 16140-7 for further identification.

0.2 Validation and verification of identification methods of microorganisms

The procedure described in this document is intended for validation of identification methods of microorganisms. This procedure comprises two parts, a performance characteristics study and an interlaboratory study.

The procedure for validation of identification methods of microorganisms in a single laboratory is described in ISO 16140-4. The procedure for verification of identification methods of microorganisms in a single laboratory is described in ISO 16140-3.

Microbiology of the food chain — Method validation —

Part 7: Protocol for the validation of identification methods of microorganisms

1 Scope

This document specifies the general principle and the technical protocol for the validation of identification methods of microorganisms for microbiology in the food chain. As there is no reference method, this document provides a protocol to evaluate the performance characteristics and validate the method workflow using well-defined strains. When required, an additional identification method can be used.

This document is applicable to the validation of identification methods of microorganisms that are used for the analysis of microorganisms in:

— 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.

Validated identification methods cannot be used instead of confirmation described in:

— the reference method;

— an alternative method validated in accordance with ISO 16140-2;

— an alternative method validated in accordance with ISO 16140-6.

In these instances, the identification method shall be validated in accordance with the ISO 16140-6 to be used as a confirmation method.

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.

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

Note 1 to entry: In the context of this document, the reference value could be the assigned identity of the strain.

[SOURCE: ISO 16140-1:2016, 2.1, modified "sample" has been replaced with "strain or specimen" — Note 1 to entry has been replaced.]

3.2
accuracy
identification accuracy
closeness of agreement between an identification result and the assigned identity of the tested strain

Note 1 to entry: The concept 'identification accuracy' is related to the identity of the analyte, i.e. genus or/and species names(s).

Note 2 to entry: 'Identification accuracy" is sometimes understood as closeness of agreement with the identification result that are being attributed to the identity of the strain given by another identification method.

Note 3 to entry: Accuracy is the usual terminology used in clinical microbiology to assess the closeness of agreement between an identification result and the assigned identity of the tested strain; the selection of this terminology was used to ensure harmonization.

Note 4 to entry: As part of the accuracy, the precision and bias are assessed during the interlaboratory study.

[SOURCE: ISO 16140-1:2016, 2.2, modified — In the definition, "measured quantity value and an assigned quantity value of a measurand" has been replaced by "an identification result and an assigned identity of the tested strain", Notes 1 and 3 to entry have been modified, and Note 2 has been deleted.]

[SOURCE: Title 21 – Food and drugs – Code of Federal Regulations (21 CFR), Parts 1-58, 800-1299]

[SOURCE: Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices]

3.3
agreement
identification agreement
method under validation study provides the same identification result as the assigned, i.e. original, identification of the tested strain

3.4
assigned identity
assigned identity is the result of the microorganism displaying generally accepted molecular and/or biochemical characteristics (e.g. Bergey's Manual of Systematics of Archaea and Bacteria)

3.5
comparison algorithm
defined calculation rules used to compare the profile of the analysed strain to the database

3.6
confirmation procedure or test
procedure or test which is carried out to verify a presumptive result

Note 1 to entry: Not all methods have a confirmation procedure

Note 2 to entry: A confirmation test can provide a positive or negative result, without yielding the identity of the analyte.

[SOURCE: ISO 16140-1:2016, 2.17, modified — Note 2 has been included.]

3.7
database
library
collection of data categories and concept entry structure of an identification database

Note 1 to entry: An identification database usually gathers the phenotypic or molecular data information of several strains from the same species or genus.

3.8
deviation
identification deviation
method under validation study does not provide the same identification result as the assigned, i.e. original, identification of the tested strain

3.9
group
group of microbial ecosystems
specimens processed in a similar way, with similar intrinsic characteristics and a similar microbial ecology (e.g. culture broths)

EXAMPLE Enrichment broths.

3.10
homology
score
identity between the profile of the analysed strain and the entry(ies) in the database

Note 1 to entry: This is normally measured as % or with score value(s).

Note 2 to entry: For select identification methods (e.g. microarray), a homology score may not be obtained.

3.11
identification method
method submitted for validation
method of analysis that provides the name (identity) of the microorganism (e.g. species or higher taxonomy ranking level)

Note 1 to entry: The method can be non-proprietary or proprietary.

Note 2 to entry: The methods can be based on various principles (e.g. phenotypic and molecular principles).

Note 3 to entry: The identification of microorganisms can help in determining whether it is a safety or spoilage concern, or is a specific technological or probiotic strain, or is likely to be resistant to an inactivation treatment, for example.

3.12
identification procedure or test
procedure or test yielding the identity of the analyte (e.g. species or higher taxonomy ranking level)

Note 1 to entry: The result of the identification test cannot be considered a confirmation result if part of a reference method or validated in accordance with ISO 16140-6:2019.