SLOVENSKI STANDARD
SIST EN ISO 16140-2:2016/oprA1:2023

01-oktober-2023

Mikrobiologija v prehranski verigi - Validacija metode - 2. del: Protokol za validacijo alternativnih (lastniških) metod glede na referenčno metodo - Dopolnilo A1

Microbiology of the food chain - Method validation - Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method

Mikrobiologie der Lebensmittelkette - Verfahrensvalidierung - Teil 2: Arbeitsvorschrift für die Validierung von alternativen (urheberrechtlich geschützten) Verfahren anhand eines Referenzverfahrens

Microbiologie de la chaîne alimentaire - Validation des méthodes - Partie 2: Protocole pour la validation de méthodes alternatives (commerciales) par rapport à une méthode de référence

Ta slovenski standard je istoveten z: **EN ISO 16140-2:2016/prA1**

ICS:

07.100.30	Mikrobiologija živil	Food microbiology

SIST EN ISO 16140-2:2016/oprA1:2023 **en,fr,de**

DRAFT AMENDMENT
ISO 16140-2:2016/DAM 1

ISO/TC **34**/SC **9** Secretariat: **AFNOR**

Voting begins on: **2023-07-18** Voting terminates on: **2023-10-10**

Microbiology of the food chain — Method validation —

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

AMENDMENT 1: Revision of qualitative MCS data evaluation, RLOD calculations in the ILS, calculation and interpretation of the RT study, and inclusion of a commercial sterility testing protocol for specific products

ICS: 07.100.30

This document is circulated as received from the committee secretariat.

ISO/CEN PARALLEL PROCESSING

Reference number
ISO 16140-2:2016/DAM 1:2023(E)

COPYRIGHT PROTECTED DOCUMENT

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

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 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.

Microbiology of the food chain — Method validation —

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

AMENDMENT 1: Revision of qualitative MCS data evaluation, RLOD calculations in the ILS, calculation and interpretation of the RT study, and inclusion of a commercial sterility testing protocol for specific products

Introduction

Replace the text with the following:

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 (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 (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 0.1 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.

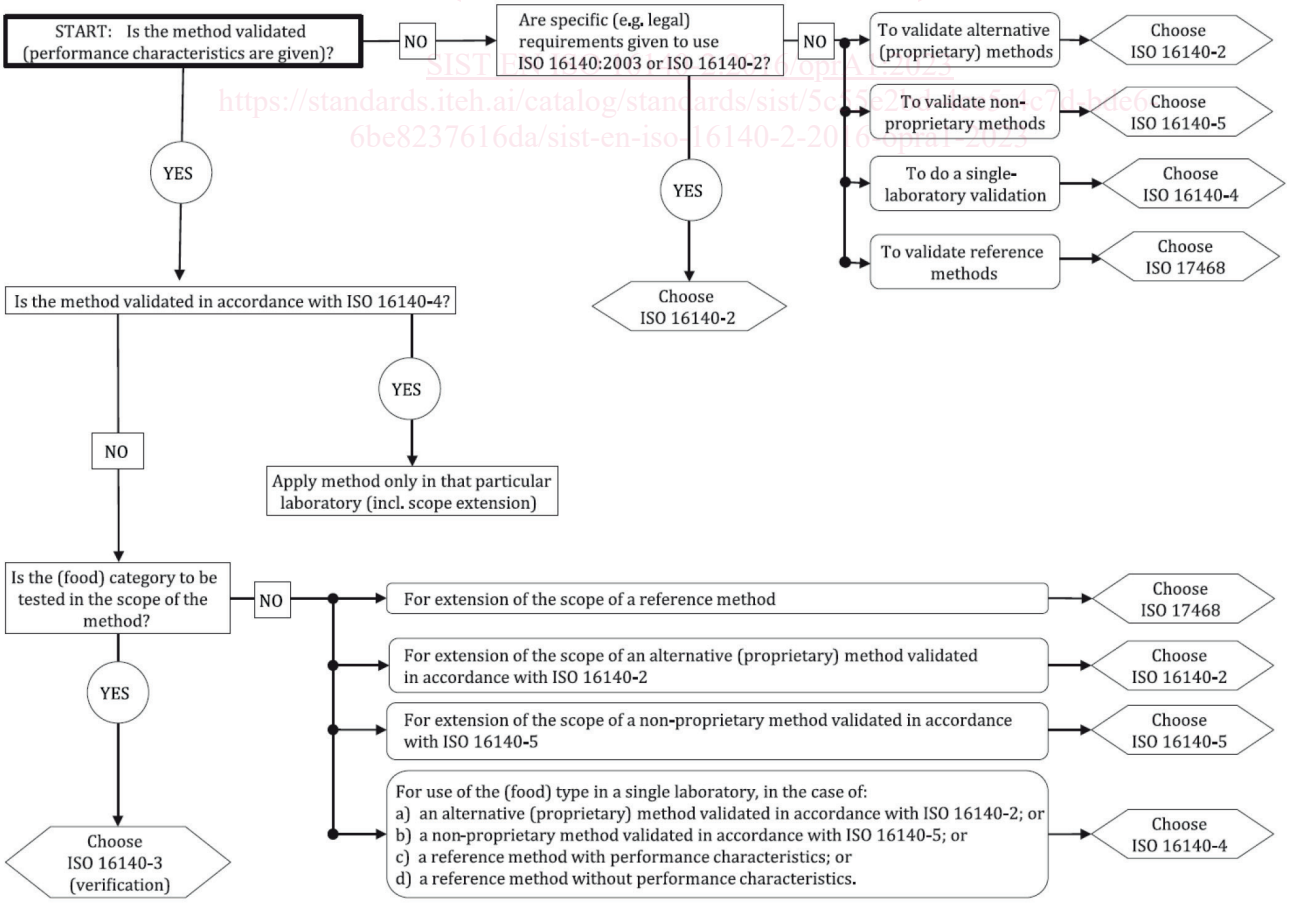

Figure 0.1 — Flow chart for application of the ISO 16140 series

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 0.2 shows the possibilities where an alternative confirmation method validated in accordance with ISO 16140-6 can be applied (see text in the boxes).

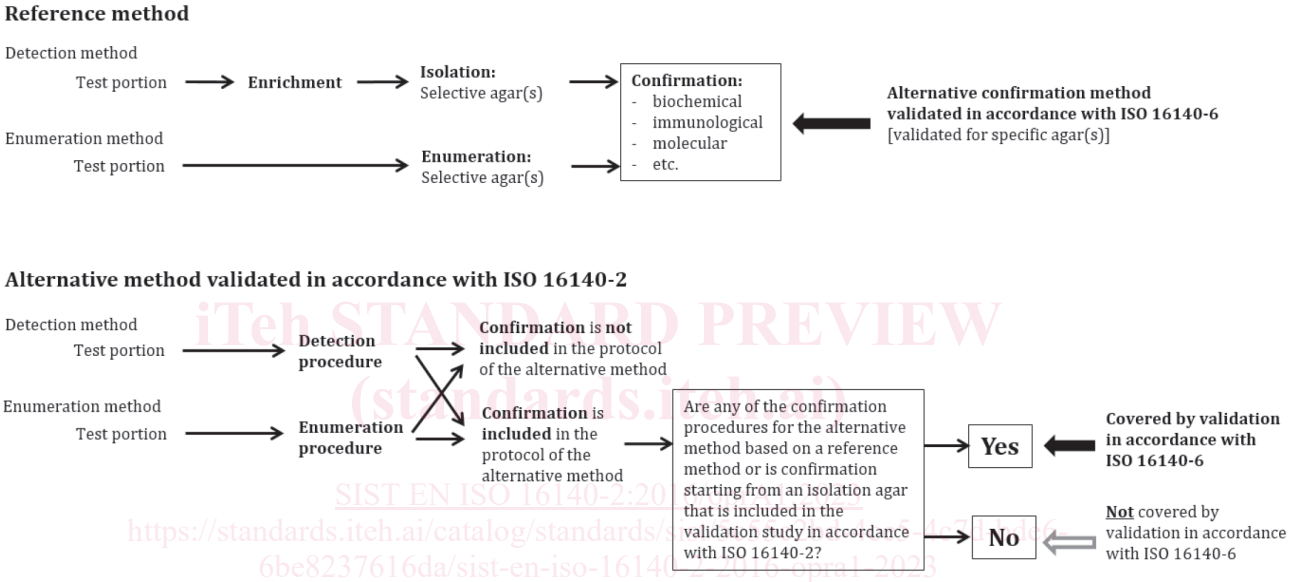

Figure 0.2 — Use of validated alternative confirmation methods (see ISO 16140-6)

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

ISO 16140-2 describes the general approach to method validation in the field of microbiology of the food chain and serves as a fundamental basis to the other parts of the ISO 16140 series, which cross-refer to it. An understanding of the performance characteristics, the (food) categories, the technical protocol and data analysis as outlined in ISO 16140-2, will provide support in the application of the ISO 16140 series in general.

Clause 4

Add the following text at the end of the clause:

For the validation of an alternative qualitative method, a corresponding qualitative reference method is selected for carrying out the validation study. This is commonly done using test portions of 10 gram, 25 gram or higher. In some cases it can be of interest to validate a qualitative alternative method against a quantitative reference method, using smaller test portion sizes.

EXAMPLE 1 *Enterobacteriaceae* criterion for pasteurized milk and other liquid pasteurized products in REGULATION (EC) No 2073/2005 is < 10 cfu/ml and refers to the quantitative method ISO 21528-2.

In such situations, it is of interest to validate the performance of qualitative alternative methods against the specified (quantitative) reference method. To that end, the technical protocol for the validation of qualitative methods (Clause 5) is to be used. For such validation study, the quantitative results of the reference method have to be converted into qualitative results prior to interpretation according to Clause 5.

EXAMPLE 2 When one or more colonies are observed on a plate using 1 ml of a 10^{-2} dilution this result corresponds to a positive detection in 0,01 gram.

NOTE Annex J provides the special case of validation of a method for commercial sterility testing of sterilized or Ultra High Temperature (UHT) dairy and plant-based liquid products.

If a technical change in a validated alternative (proprietary) method is evaluated as being major, a re-validation of this alternative method in accordance with ISO 16140-2 is needed.

When the re-validation of the alternative method is conducted, the impact on the performance characteristics shall be evaluated to determine if the changes are to be regarded as major (performance characteristics have substantially changed) or minor (no impact on performance characteristics observed). In certain cases, a major technical change in the method can be considered to be minor, if the re-validation study shows that it has no significant impact on the performance characteristics or test results. A major (technical) change that, after re-validation, has a major impact on the performance characteristics of the alternative method, requires re-verification of the method by the user laboratory in accordance with ISO 16140-3.

5.1.1

Add the following text at the end of the subclause:

The organizing laboratory shall be competent to perform both the reference method as well as the alternative method.

NOTE Competence can be demonstrated in different ways, e.g. for the reference method an ISO 17025 accreditation and for the alternative method a documented training.

5.1.2

Add the following text at the end of the subclause:

NOTE When the reference and alternative methods are based on two different principles and are performed with the same test portion, but do not share a common enrichment procedure, an unpaired data study is considered. For example, when a qualitative alternative method is validated against a quantitative reference method at a limit of 100 cfu/g. In this case a suspension of the (food) item can be used to inoculate both culture media for the reference method and the alternative method before any enrichment/multiplication of the microorganism.

5.1.3.3

Add the following text at the end of the subclause:

The alternative method shall be evaluated for a defined test portion size (e.g. 25 g, 200 g, 375 g) during the validation study. The method is considered to be validated for any test portion size up to the validated test portion size if the testing protocol (dilution ratio, incubation time and incubation temperature) is the same as that used during the validation study.

EXAMPLE 1 A reference method used in an ISO 16140-2 validation study was validated for a 'broad range of foods' using 25 g test portion and a 1:10 dilution ratio. The alternative method was validated for the category of "Raw meat and ready to cook meat products (except poultry)" using 375 g test portion and 1:10 dilution ratio at a determined incubation time. In practice, a user laboratory can use the alternative method for the "Raw meat and ready to cook meat products (except poultry)" with test portion sizes up to 375 g with a 1:10 dilution ratio at a validated incubation time (unless stated differently by the organization involved in the method validation).

EXAMPLE 2 A reference method used in an ISO 16140-2 validation study was validated for a 'broad range of foods' using 25 g test portion and a 1:10 dilution ratio. The alternative method was validated for a 'broad range of foods' using 25 g test portion and a 1:5 dilution ratio. In practice, a user laboratory can use the alternative method for all food items (broad range of foods) using a test portion size up to 25 g test portion and a 1:5 dilution ratio (unless stated differently by the organization involved in the method validation).

5.1.3.4

Add the following text before the last sentence of the second paragraph:

The interpretation of the results (positive agreement, negative agreement, etc.) is based on a comparison of the reference method result (column 1 in Tables 1 and 2) and the alternative method result, including any confirmations as described in the alternative method protocol, (column 2 in Tables 1 and 2). When positive or negative deviations are obtained, a footnote should be included at the end of each table to provide additional explanations for the interpretation of the deviations. The footnotes indicate if the result is due to a false positive or false negative result of the alternative method. The footnote is a comparison of the results of the alternative method (including any confirmations as described in the alternative-method protocol) (column 2 in Tables 1 and 2) and the confirmed alternative method (by any means) (column 3 in Tables 1 and 2).

5.1.3.4, after the second paragraph

Replace the text with the following:

Table 1 — Comparison and interpretation of sample results between the reference and alternative methods for a paired study

Result of the (reference or alternative) method per sample			
Reference method	**Alternative method** (including any confirmations as described in the alternative-method protocol)	**Confirmed alternative method** (by any means)[a]	**Interpretation** (based on the confirmed alternative-method result)
+	+	Not needed[b]	Positive Agreement (PA)
-	-	Not needed[b]	Negative Agreement (NA)
+	-	Not needed[b]	Negative Deviation due to false negative alternative-method result ($ND_{FN(alt)}$)
-	+	+	Positive Deviation (PD)
-	+	-	Positive Deviation due to false positive alternative-method result ($PD_{FP(alt)}$)[c]

[a] Confirmation of the alternative-method result is done according to 5.1.3.3.

[b] No need for additional confirmation test(s). Confirmed alternative-method result is the same as the alternative-method result.

[c] This false-positive result (FP) shall also be used to calculate the false positive ratio.

Table 2 — Comparison and interpretation of sample results between the reference and alternative methods for an unpaired study

Result of the (reference or alternative) method per sample			
Reference method	**Alternative method** (including any confirmations as described in the alternative-method protocol)	**Confirmed alternative method** (by any means)[a,b]	**Interpretation** (based on the confirmed alternative-method result)
+	+	+	Positive Agreement (PA)
+	+	-	Positive Agreement due to false positive alternative-method result ($PA_{FP(alt)}$)[c]
-	-	-	Negative Agreement (NA)
-	-	+	Negative Agreement due to false negative alternative-method result ($NA_{FN(alt)}$)
+	-	-	Negative Deviation (ND)
+	-	+	Negative Deviation due to false negative alternative-method result ($ND_{FN(alt)}$)
-	+	+	Positive Deviation (PD)
-	+	-	Positive Deviation due to false positive alternative-method result ($PD_{FP(alt)}$)[c]

[a] Confirmation of the alternative-method result is done according to 5.1.3.3

[b] Confirmation by any means is only required when the result of the alternative method does not produce viable organisms. This will be used as the confirmed alternative method result in comparison to the reference method result.

[c] These false-positive results (FP) shall also be used to calculate the false positive ratio.

Determine the Total Negative Deviation (TND) and Total Negative Agreement (TNA) for the validation study.