SLOVENSKI STANDARD
oSIST prEN ISO 16140-3:2018

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Mikrobiologija v prehranski verigi - Validacija metode - 3. del: Protokol za preverjanje referenčnih in validiranih alternativnih metod, izvedenih v posameznem laboratoriju (ISO/DIS 16140-3:2017)

Microbiology of the food chain - Method validation - Part 3: Protocol for the verification of reference and validated alternative methods implemented in a single laboratory (ISO/DIS 16140-3:2017)

Mikrobiologie von Lebensmitteln und Futtermitteln - Verfahrensvalidierung - Teil 3: Arbeitsvorschrift für die Prüfung von Referenz- und alternativer Verfahren in einem einzelnen Labor

Microbiologie de la chaîne alimentaire - Validation des méthodes - Partie 3: Protocole pour la vérification de méthodes de référence et alternatives validées appliquées dans un laboratoire (ISO/DIS 16140-3:2017)

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Microbiology of the food chain — Method validation —

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

Microbiologie de la chaîne alimentaire — Validation des méthodes —

Partie 3: Protocole pour la vérification de méthodes de référence et alternatives validées appliquées dans un laboratoire

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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).

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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*[2], 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 words ‘category’, ‘type’ and ‘item’ are 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 16140-3.

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.

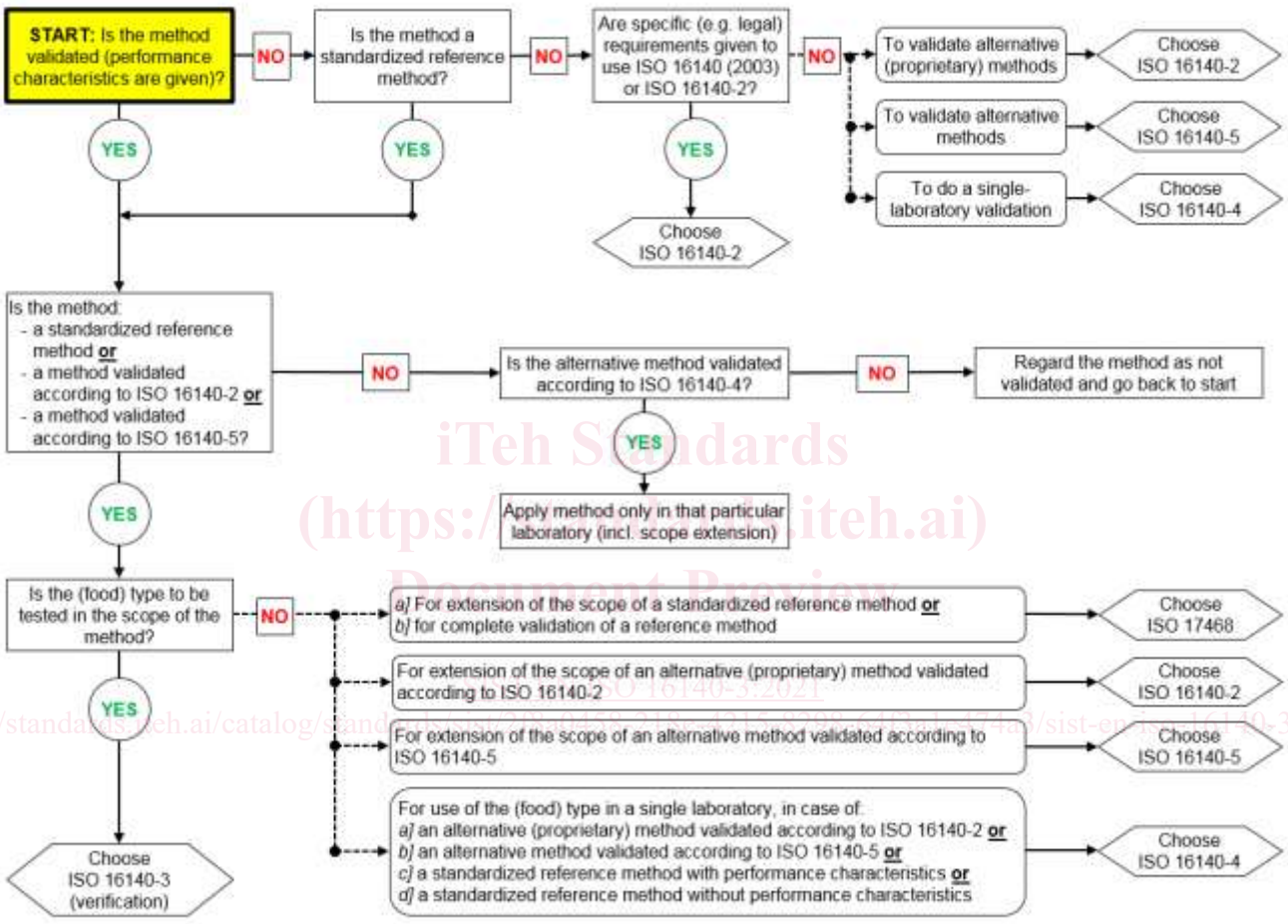

Figure 1 — Flow chart for application of the different parts of the ISO 16140 series

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.

Microbiology of the food chain — Method validation — Part 3: Protocol for the verification of reference and validated alternative methods implemented in a single laboratory

1 Scope

This part of ISO 16140 describes the protocol for the verification of reference methods, standardized reference methods and validated alternative methods for implementation in the user laboratory. Method verification does not apply to non-validated alternative methods.

This part of ISO 16140 is applicable to the verification of methods used for the analysis (detection and/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.

This part of ISO 16140 is, in particular, applicable to bacteria and fungi. Some clauses can be applicable to other (micro)organisms or their metabolites, to be determined on a case-by-case basis.

The verification focuses on those (food) items that are within the scope of validation and are tested in the user laboratory.

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 6887 (all parts), *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

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*

ISO 16140-2:2016, *Microbiology of the food chain — Method validation — Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method*

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
bias
measurement bias
estimate of a systematic measurement error, or the systematic difference between the quantitative assigned value and the average of measurement replicate results

[SOURCE: ISO 16140-1:2016, 2.9]

3.2
category
group of (food) types of the same origin

EXAMPLE Heat-processed milk and dairy products.

Note 1 to entry: The (food) categories are listed in Annex A of this document.

[SOURCE: ISO 16140-1:2016, 2.11, modified]

3.3
estimated bias (eBias)
determination of the bias based on the experimental design described in this document

Note 1 to entry: An accurate determination of the bias is not possible as the number of samples tested is small. Therefore, the term estimated bias (eBias) is used in this document.

3.4
estimated LOD_{50} ($eLOD_{50}$)
determination of the LOD_{50} (level of detection at 50 % probability of detection) based on the experimental design described in this document

Note 1 to entry: An accurate determination of the LOD_{50} is not possible as the number of samples tested is small. Therefore, the term estimated LOD_{50} ($eLOD_{50}$) is used.

Note 2 to entry: LOD_{50} is defined in ISO 16140-1:2016, 2.35.

3.5
item
single specified food, feed, environmental, or primary production matrix

EXAMPLE Food category: heat-processed milk and dairy products; food type: pasteurised dairy product; food item: crème brûlée.

[SOURCE: ISO 16140-1:2016, 2.34]

3.6
laboratory sample
sample prepared for sending to the laboratory and intended for inspection or testing

[SOURCE: ISO 6887-1:2017, 3.1]

3.7
matrix (product)
all the components of the sample

[SOURCE: ISO 16140-1:2016, 2.38]

3.8
reference material
material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process

Note 1 to entry: Properties can be quantitative or qualitative, e.g. identity of substances or species.

Note 2 to entry: Uses may include the calibration of a measurement system, assessment of a measurement procedure, assigning values to other materials, and quality control.

[SOURCE: ISO Guide 30:2015, 2.1.1, modified]

3.9
scope of laboratory application
analytes, matrices, and concentrations for an analytical method that a user laboratory claims to be capable of satisfactorily testing in its laboratory

Note 1 to entry: A method may have been validated to a broader range (scope) of analytes, matrices and concentrations than the scope that will be claimed by a user laboratory. The scope of laboratory application is ≤ the scope of validation.

3.10
scope of validation
analytes, matrices and concentrations for which a validated method of analysis can be used satisfactorily

[SOURCE: ISO 16140-1:2016, 2.70]

3.11
test portion

measured (volume or mass) representative sample taken from the laboratory sample for use in the preparation of the initial suspension

Note 1 to entry: Sometimes preparation of a test sample from the laboratory sample is required before the test portion is taken, but this is infrequently used in microbiological examinations.

[SOURCE: ISO 6887-1:2017, 3.5, modified]

3.12
test sample
sample prepared from the laboratory sample according to the procedure specified in the test method and from which test portions are taken

Note 1 to entry: Preparation of the laboratory sample before the test portion is taken is infrequently used in microbiological examinations.

[SOURCE: ISO 6887-1:2017, 3.4, modified]

3.13
type
for a given category, a group of (food) items processed in a similar way, with similar intrinsic characteristics and a similar microbial ecology

EXAMPLE Food category: heat-processed milk and dairy products; food type: pasteurised dairy product.

[SOURCE: ISO 16140-1:2016, 2.78, modified]

3.14
user laboratory
laboratory which implements a validated alternative method and/or a reference method

3.15
verification
demonstration that a validated method performs in the user's hands according to the method's specifications determined in the validation study and is fit for its intended purpose

4 Principle

4.1 General

Before performing method verification, it is crucial to refer to the published validation data for the method in order to confirm the scope of validation and to select the appropriate (food) items.

The verification is undertaken in two parts:

— implementation verification;

— (food) item verification.

User laboratories intending to verify methods that have published validation data for comparison (this includes all validated alternative methods) shall perform both the implementation and the (food) item verification. Some reference methods may *not* have published validation data. For those methods, the user laboratory will only perform (food) item verification.

Alternative methods that have not been validated shall first be validated, before a user laboratory can verify its use in their laboratory (see Figure 1).

4.2 Implementation verification

Implementation verification aims to demonstrate the competence of the user laboratory to perform the validated method. It compares user laboratory performances to those obtained during the validation. Implementation verification applies only to the following methods with published validation data:

— reference methods; and

— validated alternative methods.

The user laboratory shall:

— review the published validation data for the method;

— select one (food) item tested during the validation study that belongs within the scope of laboratory application of the user laboratory, if possible, and;

— use this (food) item and the sample size used in the validation study to perform implementation verification.

4.3 (Food) item verification

(Food) item verification applies to reference methods, with and without published validation data, and validated alternative methods with validation data. The (food) item verification sets out to demonstrate the competence of the user laboratory to perform the validated method with (food) items that are tested in the user laboratory.

4.4 Implementation verification and (food) item verification

Table 1 provides guidance on when to use implementation verification and when to use (food) item verification.

Table 1 — Implementation verification and (food) item verification

	Method with published validation data		Method without published validation data	
	Reference method	**Alternative method**	**Reference method**	**Alternative method**
Implementation verification	✓	✓	Not applicable	Not applicable [a]
(Food) item verification	✓	✓	✓	Not applicable [a]

[a] Not applicable: the method shall first be validated to ISO 16140-2 or ISO 16140-5 (see Figure 1) after which validation data are available.

Figure 2a to Figure 2d show the number of (food) items required for implementation verification and (food) item verification under different circumstances.

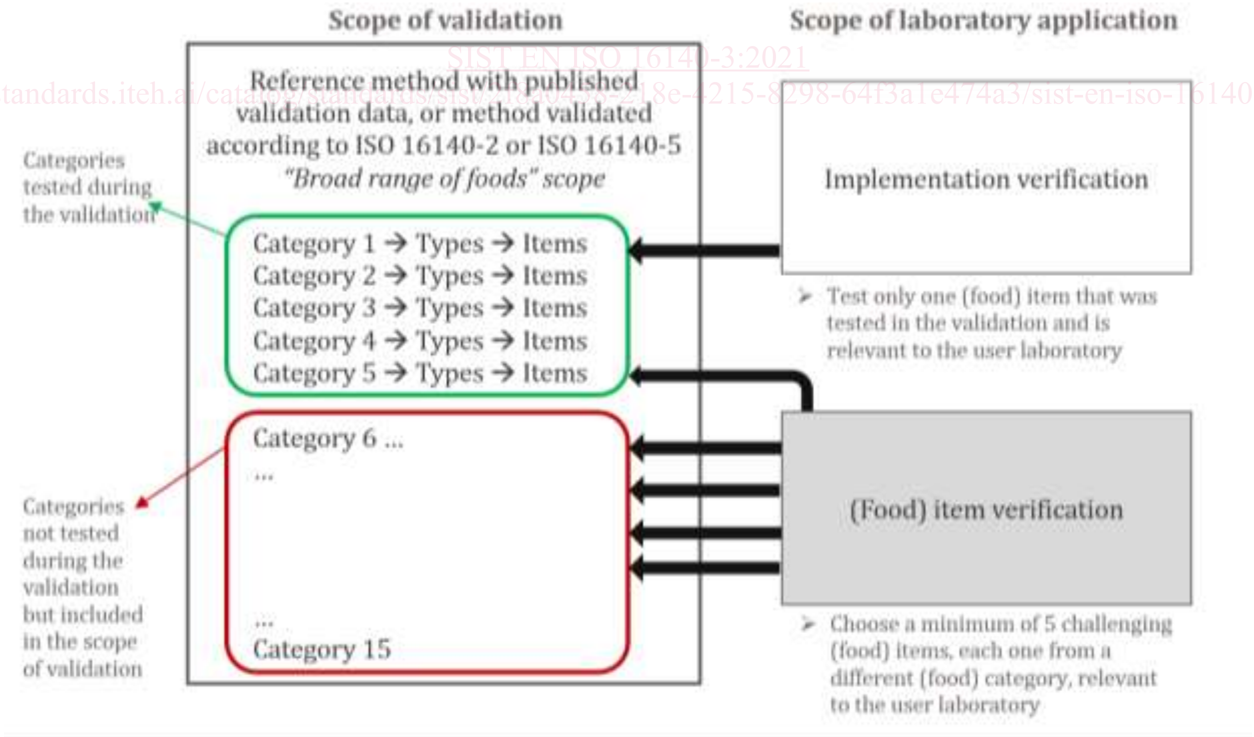
Scope of validation
Scope of laboratory application
Reference method with published validation data, or method validated according to ISO 16140-2 or ISO 16140-5
"Broad range of foods" scope
Categories tested during the validation
Category 1 → Types → Items
Category 2 → Types → Items
Category 3 → Types → Items
Category 4 → Types → Items
Category 5 → Types → Items
Category 6 ...
...
...
Category 15
Categories not tested during the validation but included in the scope of validation
Implementation verification
Test only one (food) item that was tested in the validation and is relevant to the user laboratory
(Food) item verification
Choose a minimum of 5 challenging (food) items, each one from a different (food) category, relevant to the user laboratory

Figure 2a — (Food) items required when verifying a reference method with published validation data or a validated alternative method for a "broad range of foods" scope

