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Microbiology of the food chain — Method validation —

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

Microbiologie de la chaîne alimentaire — Validation des méthodes —

Partie 4: Protocole pour la validation de méthodes internes dans un laboratoire

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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).

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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 16140-4.

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.

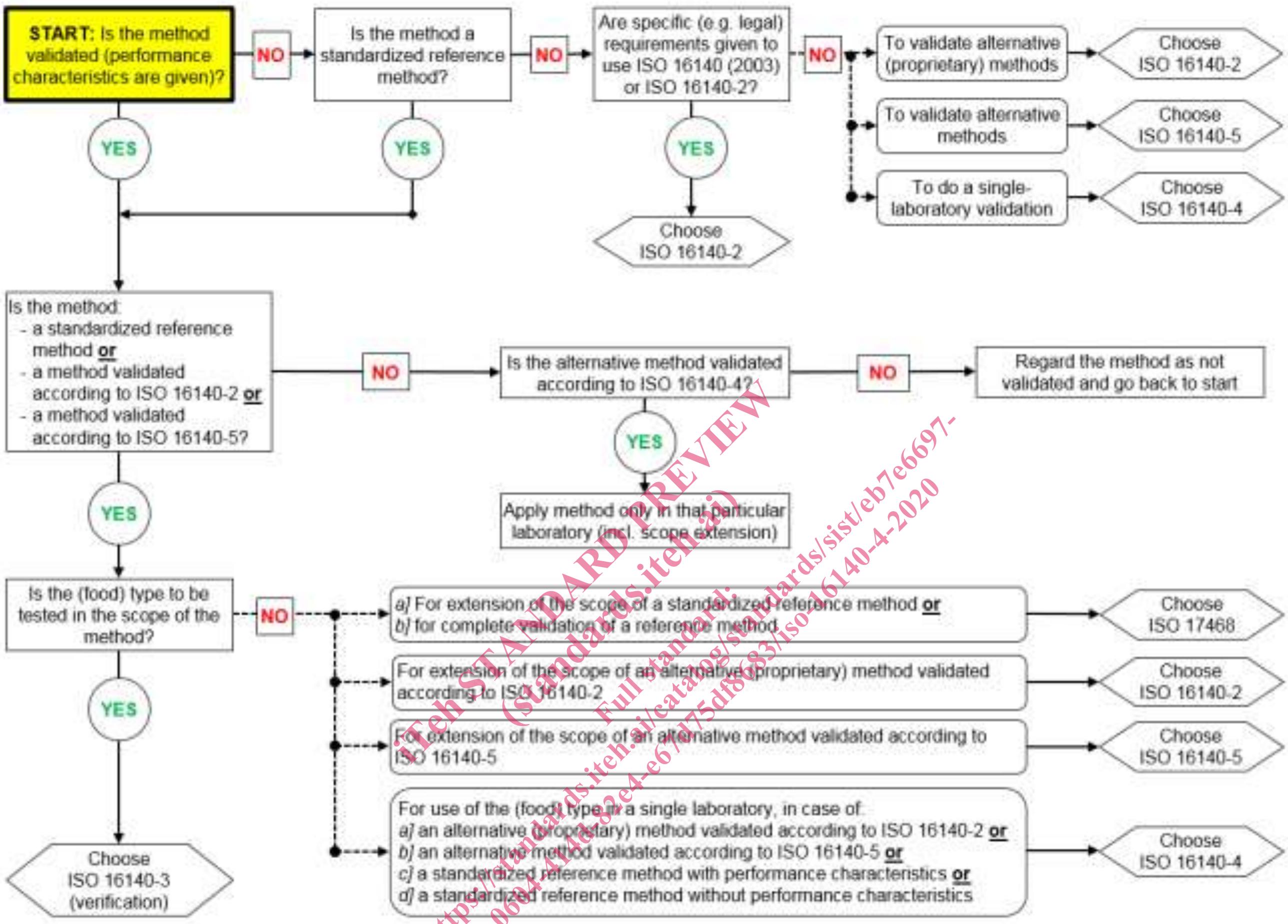
START: Is the method validated (performance characteristics are given)?
NO
Is the method a standardized reference method?
NO
Are specific (e.g. legal) requirements given to use ISO 16140 (2003) or ISO 16140-2?
NO
To validate alternative (proprietary) methods
Choose ISO 16140-2
To validate alternative methods
Choose ISO 16140-5
To do a single-laboratory validation
Choose ISO 16140-4
YES
YES
YES
Choose ISO 16140-2
Is the method:
- a standardized reference method or
- a method validated according to ISO 16140-2 or
- a method validated according to ISO 16140-5?
NO
Is the alternative method validated according to ISO 16140-4?
NO
Regard the method as not validated and go back to start
YES
Apply method only in that particular laboratory (incl. scope extension)
YES
Is the (food) type to be tested in the scope of the method?
NO
a) For extension of the scope of a standardized reference method or
b) for complete validation of a reference method
Choose ISO 17468
For extension of the scope of an alternative (proprietary) method validated according to ISO 16140-2
Choose ISO 16140-2
For extension of the scope of an alternative method validated according to ISO 16140-5
Choose ISO 16140-5
For use of the (food) type in a single laboratory, in case of:
a) an alternative (proprietary) method validated according to ISO 16140-2 or
b) an alternative method validated according to ISO 16140-5 or
c) a standardized reference method with performance characteristics or
d) a standardized reference method without performance characteristics
Choose ISO 16140-4
YES
Choose ISO 16140-3 (verification)

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.

An interlaboratory study, according to ISO 16140-2 (proprietary methods), requires at least 8 laboratories for quantitative methods and 10 laboratories for qualitative methods. ISO 16140-5 is intended to be used for interlaboratory studies comprising 4-7 laboratories for quantitative methods and 4-9 laboratories for qualitative methods. ISO 16140-5 can only be used for non-proprietary methods. Table 1 provides an overview of the different protocols.

Table 1 — Overview of different validation protocols described in ISO 16140

Number of laboratories	With reference method	Without reference method

1	Part 4 of ISO 16140: factorial or conventional	Part 4 of ISO 16140: factorial or conventional
4 to 7 (quantitative method)/ 4 to 9 (qualitative method)	Part 5 of ISO 16140: for non-proprietary methods only	Part 5 of ISO 16140: for non-proprietary methods only
≥ 8 (quantitative method)/ ≥ 10 (qualitative method)	Part 2 of ISO 16140 (for the interlaboratory study part)	Not available

The aim of the single-laboratory validation studies described in this part of ISO 16140 is to assess the performance of a method within a single laboratory, typically across a number of (food) categories and (food) types. The protocols in this part of ISO 16140 only validate the method for the particular laboratory. A generalization to other laboratories is not within the scope of these protocols. However, extension to other laboratories is possible if ISO 16140-4 is used as the first phase of validation, followed by an interlaboratory study as described in ISO 17468[2].

The general principles and concepts for single-laboratory validations are the same as those described in ISO 16140-2 for the validation of alternative (proprietary) methods against a reference method. Part 4 cannot be used without ISO 16140-2, as many definitions and procedures are given in part 2 of ISO 16140. In addition to the validation parameters described in ISO 16140-2, part 4 of ISO 16140 describes the calculation of in-house repeatability and in-house reproducibility. Calculation of these parameters is not required if an interlaboratory study is to be conducted after the single-laboratory validation (i.e. if the single-laboratory validation is only the first phase of validation).

This part of ISO 16140 provides two strategies for the single-laboratory method validation, using one or more strains of the target organism. The first strategy is based on a factorial plan while the second strategy provides method comparison designs derived from the protocols of ISO 16140-2 together with protocols for the determination of the in-house reproducibility. Protocols are provided for qualitative and quantitative methods with, and without, reference methods.

Factorial experiments require more experimental control and planning, but involve a smaller number of experiments compared to the conventional approach, while at the same time providing more information about the sources of variation. The factorial design offers several advantages. Factorial approach takes into account the conditions a laboratory encounters during routine testing and provides more information on the factors that vary within a laboratory (personnel, culture media, etc.) across relevant (food) matrices, while using fewer samples to assess the performance of the method. In short, it allows greater efficiency: fewer test results are required in order to obtain comparable levels of reliability.

Different (food) types are included and all identified influence factors are explicitly taken into consideration and systematically varied across their respective ranges. The design offers assessment of the precision of quantitative methods. It allows computation of reliable and representative single-laboratory validation parameters such as in-house reproducibility standard deviation, LOD_{50} or RLOD values because it provides information on the variability of these values under different measurement conditions. This greatly enhances the value of the validation.

In short, it allows greater efficiency: fewer test results are required in order to obtain comparable levels of reliability.

If a reference method is available, the validation of a method is conducted by comparing the method to the reference method. This allows inclusion of naturally contaminated samples in the validation process and thus provides a more realistic picture of the performance of the method. If no reference method is

available, the validation process is based on artificially contaminated samples only. Part 4 of ISO 16140 provides protocols for both situations.

Microbiology of the food chain — Method validation — Part 4: Protocol for single-laboratory (in-house) method validation

1 Scope

This part of ISO 16140 describes the protocols for single-laboratory validation of methods for microbiology in the food chain. The protocols in this part of ISO 16140 only validate the method for the laboratory conducting the study.

This part of ISO 16140 is applicable to single-laboratory validation of 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.

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.

Single-laboratory validation is required if an interlaboratory validation according to ISO 16140-2 is not appropriate, e.g. for in-house methods. Possible applications are:

— validation of new in-house method;

— the first step in the validation process according to ISO 17468[2];

— extension of the scope of an ISO 16140-2 validated method: e.g. category extension or test portion size;

— modifications of existing methods.

Within ISO 17468[2], single-laboratory validation is the first step in the standardization of a method. It can be applied only for methods that are fully specified with regard to all relevant parameters (including tolerances on temperatures and specifications on culture media) and which have already been optimised.

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 (series), *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
block
group of settings which have to be conducted in parallel or in a short time interval, and which are used for the same samples

EXAMPLE Block = settings conducted in parallel =
Technician 'a' + culture medium 'b' + temperature 'a' + incubator 'a'
AND
Technician 'b' + culture medium 'a' + temperature 'b' + incubator 'b'

3.2
factor
qualitative or quantitative parameter within the method that can be varied at two or more levels within the limits of the specified method

EXAMPLE Technician.

Note 1 to entry: In this part of ISO 16140, only those factors that are in line with the prescription of the method are considered.

3.3
factor level
value of the factors within the experimental design

EXAMPLE Technician 'a', Technician 'b', etc.

Note 1 to entry: In this part of ISO 16140, each factor is varied at two factor levels, 'a' and 'b'.

3.4
in-house repeatability
measurement precision under a set of in-house repeatability conditions of measurement in a particular laboratory

Note 1 to entry: In-house repeatability conditions include the same measurement procedure, same technicians, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period of time in a particular laboratory.

3.5
in-house reproducibility
measurement precision under a set of in-house reproducibility conditions of measurement in a particular laboratory

Note 1 to entry: In-house reproducibility conditions include different technicians, operating conditions, and replicate measurements on the same or similar objects over a longer period of time in a particular laboratory.

3.6
level of detection
LOD_x
‹qualitative methods› measured analyte concentration, obtained by a given measurement procedure, for which the probability of detection is *x*

EXAMPLE LOD_{50} is the level of detection for which 50 % of tests give a positive result.

Note 1 to entry: The term 'level of detection' is used for qualitative methods in microbiology based on replicate analyses with three different inoculation levels of the target analyte in a tested matrix. The replicates are analysed, and the number of positive results is recorded (e.g. 20 %, 70 % and 100 %) respectively at each inoculation level. These data are then used to determine the number of cells that would give 50 % positive using a generalised linear model (see ISO 16140-2). This differs from the procedure used for chemical and physical methods for which a 'limit of detection' is defined as the lowest quantity of an analyte that can be distinguished from the absence of that analyte with a stated confidence level.

[SOURCE: ISO 16140-1:2016, 2.35]

3.7
limit of quantification
LOQ
limit of determination
‹quantitative methods› the lowest analyte concentration that can be quantified with an acceptable level of precision and trueness under the conditions of the test

[SOURCE: ISO 16140-1:2016, 2.36]

3.8
probability of detection
POD
proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration

Note 1 to entry: For qualitative methods, POD represents the probability of detection.

[SOURCE: ISO 16140-1:2016, 2.53, modified]

3.9
relative level of detection
RLOD
level of detection at P = 0,50 (LOD_{50}) of the alternative (proprietary) method divided by the level of detection at P = 0,50 (LOD_{50}) of the reference method

Note 1 to entry: For purposes of alternative-method acceptance, the derived RLOD is checked with the acceptability limit for conformity.

[SOURCE: ISO 16140-1:2016, 2.61]

3.10
single-laboratory method validation
in-house method validation
establishment of the performance characteristics of a method for the one particular laboratory in which the validation is conducted

3.11
setting
combination of factor levels

EXAMPLE Technician 'a' + culture medium 'b' + temperature 'a' + etc.

Note 1 to entry: These conditions can be described by the combination of levels of factors varied within the study.

4 General principles of the single-laboratory method validation

4.1 General

A single-laboratory method validation study is the first step in the framework of general method validation and is needed to assess the performance of the method across a large number of (food) types and (food) items. The second step is an interlaboratory study to assess the performance of the method across the required number of laboratories. A single-laboratory method validation study is used to demonstrate the performance of the method in the laboratory that conducted the study. The results are only valid for that particular laboratory.

This part of ISO 16140 describes two approaches for single-laboratory validation.

— Factorial approach:
  — performance measures derived from ISO 16140-2;
  — orthogonal, factorial study design;
  — more routine settings covered, less tests than conventional approach required.
— Conventional approach:
  — performance measures derived from ISO 16140-2;
  — step-wise procedure;
  — study design derived from ISO 16140-2.

Validation procedures are dependent on whether the method is qualitative or quantitative, and on whether a factorial or a conventional approach is chosen. The factorial single-laboratory validation approach can only be used for a fully developed and optimised method. A conventional approach investigates the method for one specific setting (that is one set of specific conditions under which the method is performed). The main differences in approach for the single-laboratory validation covered in this document are the number of various (food) items and the number of tests required to show that the method performs adequately. Validation of methods with, and without, a reference method is possible with the proposed protocols.

The scope of the validation procedure shall be determined at the start of the process, e.g.: validation of in-house methods, the first step in the validation process according to ISO 17468[2], extension of the scope of an ISO 16140-2 validated method, modification of existing methods.

For methods that include a PCR detection step, an assessment of the performance characteristics for the PCR based detection step is described in ISO 22118[3]. It is recommended, to start with the assessment of the relevant performance parameters of the PCR step first (based on ISO 22118[3]), followed by a

