SLOVENSKI STANDARD
SIST EN ISO 16140-3:2021/oprA1:2025

01-februar-2025

Mikrobiologija v prehranski verigi - Validacija metode - 3. del: Protokol za preverjanje referenčnih in validiranih alternativnih metod, izvedenih v posameznem laboratoriju - Dopolnilo A1: Validirane metode identifikacije mikroorganizmov - Tehnični protokol za preverjanje (ISO 16140-3:2021/DAmd1:2024)

Microbiology of the food chain - Method validation - Part 3: Protocol for the verification of reference methods and validated alternative methods in a single laboratory - Amendment 1: Validated identification methods of microorganisms - Technical protocol for verification (ISO 16140-3:2021/DAmd1:2024)

Mikrobiologie der Lebensmittelkette - Verfahrensvalidierung - Teil 3: Arbeitsvorschrift für die Verifizierung von Referenz- und validierten alternativen Verfahren in einem Einzel-Labor - Änderung 1: Verifizierung von validierten Identifizierungsverfahren (ISO 16140 3:2021/DAmd1:2024)

Microbiologie de la chaîne alimentaire - Validation des méthodes - Partie 3: Protocole pour la vérification dans un seul laboratoire de méthodes de référence et de méthodes alternatives validées - Amendement 1: Méthodes validées d'identification des micro-organismes - Protocole technique pour la vérification (ISO 16140-3:2021/DAmd1:2024)

Ta slovenski standard je istoveten z: **EN ISO 16140-3:2021/prA1:2024**

ICS:

07.100.30	Mikrobiologija živil	Food microbiology

SIST EN ISO 16140-3:2021/oprA1:2025 **en,fr,de**

DRAFT
Amendment

ISO 16140-3:2021/ DAM 1

Microbiology of the food chain — Method validation —

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

AMENDMENT 1: Validated identification methods of microorganisms — Technical protocol for verification

ICS: 07.100.30

ISO/TC **34**/SC **9**

Secretariat: **AFNOR**

Voting begins on:
2024-12-04

Voting terminates on:
2025-02-26

This document is circulated as received from the committee secretariat.

ISO/CEN PARALLEL PROCESSING

Reference number
ISO 16140-3:2021/DAM 1:2024(en)

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Published in Switzerland

Foreword

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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.

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Microbiology of the food chain — Method validation —

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

AMENDMENT 1: Validated identification methods of microorganisms — Technical protocol for verification

Introduction (0.1)

Replace the third sentence with:

The ISO 16140 series consists of several parts with the general title, *Microbiology of the food chain — Method validation:*

Add the following text after the sixth bullet point:

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

Replace the reference to ISO 17468:2016, 3.5, in the sixth paragraph with:

In the ISO 16140 series, reference methods include standardized reference (ISO and CEN) methods as defined in ISO 17468:2023, 3.7, as a “reference method described in a standard”.

Replace the first sentence after the NOTE 2 on Page vii with:

ISO 16140-6 and this document (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)].

Replace the verb “defines” with “specifies” in the third sentence of the next paragraph:

The validation study in ISO 16140-6 clearly specifies the selective agar(s) from which strains can be confirmed using the alternative confirmation method.

Replace Figure 2 with:

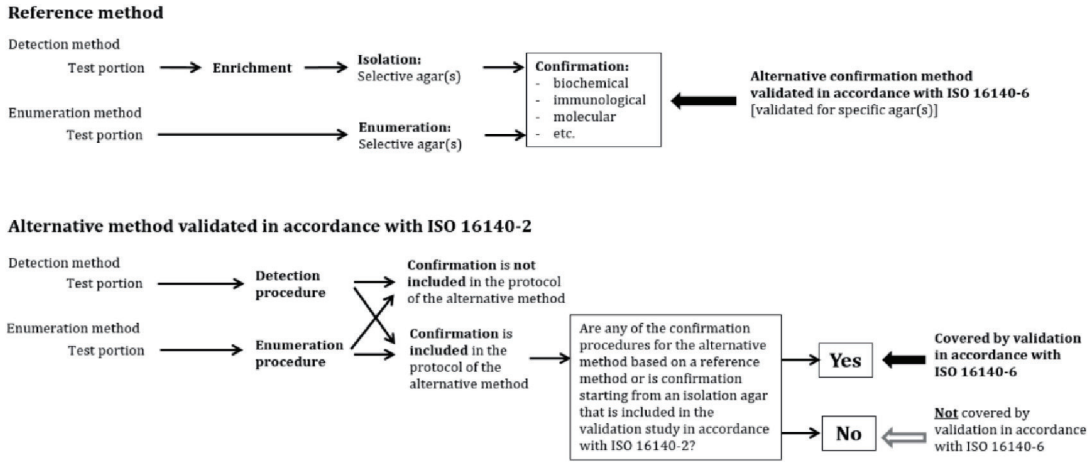

Figure 2 — Use of validated alternative confirmation methods (see ISO 16140-6)

Replace the first sentence of the EXAMPLE below Figure 2 with:

EXAMPLE 1 An example application of a validated alternative confirmation method is as follows.

Add the following text in the end of 0.1 on Page viii:

This document (ISO 16140-7) addresses the validation of identification procedures (e.g. molecular identification using multiplex PCR or DNA sequencing or mass spectrometry). ISO 16140-7 differs from the other parts in the ISO 16140 series, as it is intended for microbial identification for which there is no reference method and, therefore, it is not possible to run a method comparison study. The validation study in ISO 16140-7 specifies the identification method principle, the identification database and algorithm when appropriate, and the 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.

NOTE 3 Whole-genome sequencing (WGS) in accordance with ISO 23418 will eventually be a reference method for all microorganisms, but the implementation of this technique is still at an early stage. Therefore, the use of WGS cannot currently be requested as a reference method for a large panel of strains.

ISO 16140-7:—, 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 this document 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.

If the identification method is also 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 this document for further identification.

EXAMPLE 2 An alternative confirmation method of *Campylobacter* genus can be validated in accordance with ISO 16140-6 and compared to the mandatory confirmation procedure at the genus level described in ISO 10272-1. The identification at the *Campylobacter* species level is optional in ISO 10272-1 and ISO 10272-2 and is therefore not mandatory. In this instance, an identification method at the *Campylobacter* species level can be validated in accordance with this document.

Introduction (0.2)

Add the following text after NOTE 2 on Page ix as last bullet point:

— ISO 16140-7 for identification methods.

Clause 1

Add the following text in the last paragraph before the last sentence:

The technical protocol for the verification of validated identification methods is described in Clause 9.

Clause 3

Add the following definitions:

3.22
agreement
identification agreement
method under validation study provides the same identification result as the assigned, i.e. original, identification of the tested strain

[SOURCE: ISO 16140-7:—, 3.2]

3.23
assigned identity
result of the microorganism identification displaying generally accepted molecular and/or biochemical characteristics

EXAMPLE Bergey's Manual of Systematics of Archaea and Bacteria.[21]

[SOURCE: ISO 16140-7:—, 3.3]

3.24
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

[SOURCE: ISO 16140-7:—, 3.7]

3.25
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 for example 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.

[SOURCE: ISO 16140-7:—, 3.10]

Clause 8

Add a line to Table 16:

Identification method	Panel of strains	100 % agreement

Clause 9

Add the following text:

9 Validated identification methods — Technical protocol for verification

9.1 General

The verification of validated identification methods only requires implementation verification. The sample is an isolated colony on defined selective or non-selective agar plates.

9.2 Implementation verification

Implementation verification aims to demonstrate the competence of the user laboratory to perform the validated identification method. This is achieved by its ability to obtain the expected results on an isolated colony from specified selective or non-selective agar(s).

The user laboratory shall:

— review the validation data for the method (the validation data can be obtained from the alternative methods validation report).

— select the selective agar plate(s) tested during the validation study that belongs within the scope of the laboratory; if multiple selective agar plates were tested, a combination of all plates tested shall be used in the implementation study; whenever possible and relevant for the scope of laboratory application, test mainly the selective agar plate(s).

— use this selective agar plate(s) to perform implementation verification. If no selective agar plate was tested, select and use one non-selective agar plate tested during the validation study to perform the implementation.

NOTE 1 Selective agars may enable the recovery of microorganisms from specific clades, (i) family, (ii) genus or group of species (e.g. *Bacillus cereus* group). In this document, "group of species" is substituted with "genus" to improve readability. However, the word "genus" is interchangeable with "group of species".

NOTE 2 Detailed examples on verification of a validated identification method are given in Annex G.

9.3 Experimental design

9.3.1 General

The set of strains to be tested depends on the scope of the validated method and the scope of laboratory application. For the implementation verification, the number of strains to be tested is given in Table 17. The number of strains to be tested depends on the scope of the validated identification to be verified and the scope of the laboratory. If the identification method covers multiple families, genera and species, 15 strains shall be tested and as much as possible from different families, genera and species. If the identification method is restricted to multiple genera within one single family, 10 different strains shall be tested and as much as possible from different genera and species. If the identification method is restricted to multiple species within one single genus, 5 different strains shall be tested and as much as possible from different species.

Select test strains able to recover on the tested selective agar(s).