ICS 07.100.30 SLOVENSKÁ TECHNICKÁ NORMA Január 2026

STN	**Mikrobiológia potravinárskeho reťazca Metóda validácie Časť 4: Postup na metódu validácie v jednom (domácom) laboratóriu Zmena A2: Postup pre jedno-laboratórnu validáciu metód identifikácie mikroorganizmov (ISO 16140-4: 2020/Amd 2: 2025)**	**STN EN ISO 16140-4/A2** 56 0107

Microbiology of the food chain - Method validation - Part 4: Protocol for method validation in a single laboratory - Amendment 2: Protocol for single-laboratory validation of identification methods of microorganisms (ISO 16140-4:2020/Amd 2:2025)

Táto norma obsahuje anglickú verziu euró pskej normy.
This standard includes the English version of the European Standard.

Táto norma bola oznámená vo Vestníku ÚNMS SR č. 12/25

Obsahuje: EN ISO 16140-4:2020/A2:2025, ISO 16140-4:2020/Amd 2:2025

141347

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 16140-4:2020/A2

August 2025

ICS 07.100.30

English Version

Microbiology of the food chain - Method validation - Part 4: Protocol for method validation in a single laboratory - Amendment 2: Protocol for single-laboratory validation of identification methods of microorganisms (ISO 16140-4:2020/Amd 2:2025)

Microbiologie de la chaîne alimentaire - Validation des méthodes - Partie 4: Protocole pour la validation de méthodes dans un seul laboratoire - Amendement 2: Protocole pour la validation dans un seul laboratoire de méthodes d'identification des microorganismes (ISO 16140-4:2020/Amd 2:2025)

Mikrobiologie der Lebensmittelkette - Verfahrensvalidierung - Teil 4: Arbeitsvorschrift für die Einzellabor-Verfahrensvalidierung - Änderung 2: Arbeitsvorschrift für die Einzellabor-Verfahrensvalidierung von Identifizierungsverfahren von Mikroorganismen (ISO 16140-4:2020/Amd 2:2025)

This amendment A2 modifies the European Standard EN ISO 16140-4:2020; it was approved by CEN on 25 August 2025.

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Ref. No. EN ISO 16140-4:2020/A2:2025 E

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European foreword

This document (EN ISO 16140-4:2020/A2:2025) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 463 “Microbiology of the food chain” the secretariat of which is held by AFNOR.

This Amendment to the European Standard EN ISO 16140-4:2020 shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by February 2026, and conflicting national standards shall be withdrawn at the latest by February 2026.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

Any feedback and questions on this document should be directed to the users’ national standards body/national committee. A complete listing of these bodies can be found on the CEN website.

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Endorsement notice

The text of ISO 16140-4:2020/Amd 2:2025 has been approved by CEN as EN ISO 16140-4:2020/A2:2025 without any modification.

International Standard

ISO 16140-4

First edition
2020-07

AMENDMENT 2
2025-08

Microbiology of the food chain — Method validation —

Part 4: Protocol for method validation in a single laboratory

AMENDMENT 2: Protocol for single-laboratory validation of identification methods of microorganisms

Microbiologie de la chaîne alimentaire — Validation des méthodes —

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

AMENDEMENT 2: Protocole pour la validation dans un seul laboratoire de méthodes d'identification des microorganismes

Reference number
ISO 16140-4:2020/Amd.2:2025(en)

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Published in Switzerland

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 document 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 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.

ISO 16140-4:2020/Amd.2:2025(en)

Microbiology of the food chain — Method validation —

Part 4: Protocol for method validation in a single laboratory

AMENDMENT 2: Protocol for single-laboratory validation of identification methods of microorganisms

Introduction (0.1), first paragraph

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 list item:

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

Introduction (0.1), sixth paragraph

Replace the reference to ISO 17468:2016, 3.5, 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”.

Introduction (0.1), after Figure 1

Number the note to Note 1:

NOTE 1 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.

Introduction (0.1), tenth paragraph

Replace the first sentence with:

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)].

Replace the verb “defines” with “specifies” in the fourth sentence:

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

Introduction (0.1), Figure 2

Replace Figure 2 with:

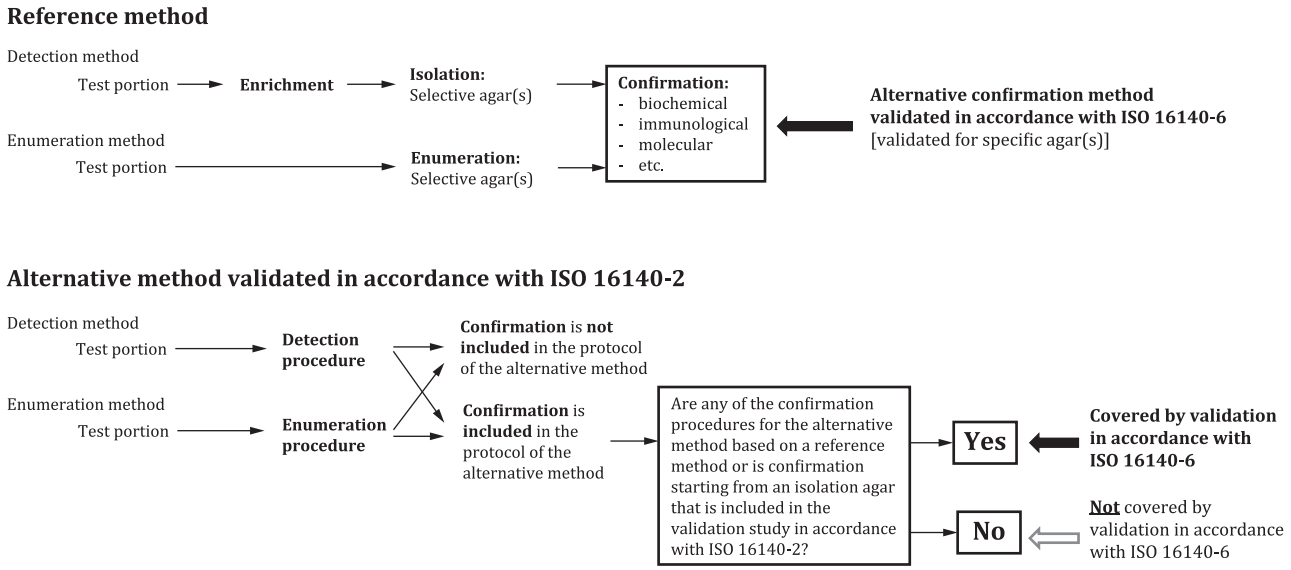

Figure 2 — Use of validated alternative confirmation methods (see ISO 16140-6)

Introduction (0.1), Example

Number the example to Example 1:

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

Introduction (0.1), after the last paragraph

Add the following text in the end of 0.1:

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 2 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 16140-7:2024, 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.

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 ISO 16140-7 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 ISO 16140-7. If the method is validated by ISO 16140-6 and ISO 16140-7, it can be used for both confirmation and identification purposes.

Introduction (0.2), after the second paragraph

Add the following text as a new paragraph after the second paragraph:

Single-laboratory validation of identification methods of microorganisms can also be performed under certain conditions: the general principles are the same as those described in ISO 16140-7 for the validation of identification methods of microorganisms (except there is no interlaboratory study). Further information is given in Annex I.

Clause 1, second paragraph

Add the following text as the last list item:

— identification methods of microorganisms (see Annex I).