

<b>STN</b>	<b>Mikrobiológia potravinárskeho reťazca Metóda validácie Časť 3: Postup na verifikáciu referenčnej metódy a validovaných alternatívnych metód zavedených v jednom laboratóriu Zmena A1: Postup na overenie validovaných metód identifikácie mikroorganizmov (ISO 16140-3: 2021/Amd 1: 2025)</b>	<b>STN EN ISO 16140-3/A1</b>  56 0107
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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: Protocol for verification of validated identification methods of microorganisms (ISO 16140-3:2021/Amd 1:2025)

Táto norma obsahuje anglickú verziu európskej 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 16140-3:2021/A1:2025, ISO 16140-3:2021/Amd 1:2025

**141346**

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Úrad pre normalizáciu, metrológiu a skúšobníctvo Slovenskej republiky, 2026  
Slovenská technická norma a technická normalizačná informácia je chránená zákonom č. 60/2018 Z. z. o technickej normalizácii v znení neskorších predpisov.



EUROPEAN STANDARD

EN ISO 16140-3:2021/A1

NORME EUROPÉENNE

EUROPÄISCHE NORM

August 2025

ICS 07.100.30

English Version

**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: Protocol for verification of validated  
identification methods of microorganisms (ISO 16140-  
3:2021/Amd 1:2025)**

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: Protocole pour la vérification des méthodes validées d'identification des micro-organismes (ISO 16140-3:2021/Amd 1:2025)

Mikrobiologie der Lebensmittelkette - Verfahrensvalidierung - Teil 3: Arbeitsvorschrift für die Verifizierung von Referenz- und validierten alternativen Verfahren in einem Einzel-Labor - Änderung 1: Validierte Identifizierungsverfahren von Mikroorganismen - Technische Arbeitsvorschrift zur Verifizierung (ISO 16140-3:2021/Amd 1:2025)

This amendment A1 modifies the European Standard EN ISO 16140-3:2021; it was approved by CEN on 25 August 2025.

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COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

**CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels**

**EN ISO 16140-3:2021/A1:2025 (E)**

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## **European foreword**

This document (EN ISO 16140-3:2021/A1:2025) 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 16140-3:2021 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.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and the United Kingdom.

## **Endorsement notice**

The text of ISO 16140-3:2021/Amd 1:2025 has been approved by CEN as EN ISO 16140-3:2021/A1:2025 without any modification.



# International Standard

**ISO 16140-3**

## **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: Protocol for verification of validated identification methods of microorganisms**

*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: Protocole pour la vérification des méthodes  
validées d'identification des micro-organismes*

**First edition  
2021-01**

**AMENDMENT 1  
2025-08**

## ISO 16140-3:2021/Amd.1:2025(en)



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**ISO 16140-3:2021/Amd.1:2025(en)****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](http://www.iso.org/directives)).

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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.

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](http://www.iso.org/members.html).

**ISO 16140-3:2021/Amd.1:2025(en)****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: Protocol for verification of validated 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), 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:

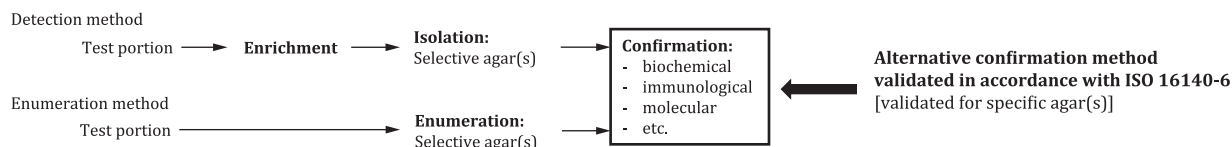
The validation study in ISO 16140-6 clearly specifies the selective agar(s) from which strains can be confirmed using the alternative confirmation method.

## ISO 16140-3:2021/Amd.1:2025(en)

## Introduction (0.1), Figure 2

Replace Figure 2 with:

## Reference method



## Alternative method validated in accordance with ISO 16140-2

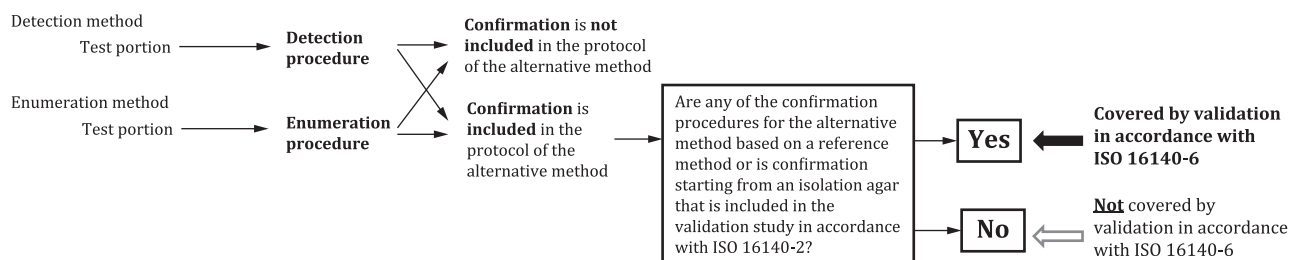


Figure 2 — Use of validated alternative confirmation methods (see ISO 16140-6)

## 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 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 16140-7:2024, 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.

**ISO 16140-3:2021/Amd.1:2025(en)**

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), second paragraph*

Add the following text as the last list item:

- ISO 16140-7 for identification methods.

*Clause 1, last paragraph*

Add the following text before the last sentence:

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

**koniec náhľadu – text ďalej pokračuje v platenej verzii STN**