



National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Update on activities in ISO and CEN

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Relevant groups in ISO and CEN

ISO/TC34/SC9:

- › ISO: International Standardisation Organisation
- › TC34: Technical Committee 34 on Food products
- › SC9: Subcommittee 9: Microbiology of the food chain

CEN/TC463:

- › CEN: European Committee for Standardisation
- › TC463: Technical Committee 463: Microbiology of the food chain

Next annual meeting: 13-17 June 2022, online





Development ISO/TS 6579-4

'Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 4: Identification of monophasic *Salmonella* Typhimurium (1,4,[5],12:i:-) by polymerase chain reaction (PCR)'.

ISO: International Standard

TS: Technical Specification

TS - Definition ISO/IEC Directives part 2, 2021:

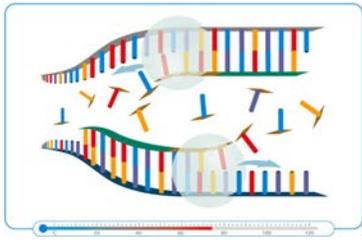
document published by ISO or IEC for which there is the future possibility of agreement on an International Standard, but for which at present

- the required support for approval as an International Standard cannot be obtained,
- there is doubt on whether consensus has been achieved,
- the subject matter is still under technical development, or
- there is another reason precluding immediate publication as an International Standard



History development ISO/TS 6579-4

- › June 2014 (annual meeting ISO, CEN): agreed to start development.
- › Nov/Dec 2014: inventory for available methods.
- › June 2015: agreed to have technical work in CEN (Burkhard Malorny).
- › March 2016: first working draft.
- › Nov 2016-March 2017: call for test strains for testing/validation.
- › April-December 2017: typing 420 isolates (obtained after call).
- › 2018: testing WD with 172 selected strains by EURL and NRL DE.
- › 2019: re-analysis strains with deviating results; reporting results.
- › March 2020: report sent to CEN/TC463-WG1 – agreed to move development to ISO/TC34/SC9-WG10.
- › May-Aug 2020: NWIP vote & call for experts in ISO/TC34/SC9.
- › Nov 2020: 1st meeting WG10.
- › April-May 2021: voting round on draft ISO/CD 6579-4.
- › June-Nov 2021: preparation draft ISO/DTS 6579-4 and proposal ILS.



Draft ISO/TS 6579-4

Describes three different PCR methods:

- PCR method 1: a probe-based multiplex real-time PCR. Primers and probes published by Maurischat et al., 2015.
- PCR method 2: an agarose gel-based multiplex PCR. Primers published by EFSA, 2010 and Tennant et al., 2010.
- PCR method 3: an agarose gel-based single target PCR. Primers published by Maurischat et al., 2015; primers internal control published by Gallien, 2003.

Method is applicable for:

- differentiation of the isolate under analysis between monophasic *Salmonella* Typhimurium and the monophasic variant of another *Salmonella* non-Typhimurium serovar;
- identification of the isolate under analysis being either monophasic *Salmonella* Typhimurium or (biphasic) *Salmonella* Typhimurium.



Interlaboratory study (ILS) ISO/TS 6579-4 - I

- For determination performance characteristics an ILS is organised.
- Design ILS based upon info of EN ISO 16140-6 ('Microbiology of the food chain – Method validation - Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures'):
 - › Per PCR method at least 10 valid data sets from at least 10 collaborators needed.
 - › A total of (at least) 24 different strains shall be tested for inclusivity (target strains) and exclusivity (non-target strains).
 - › Strains selected from 172 strains tested during method evaluation study.
 - › Results found by collaborators are compared to 'true' identity of the strains → selected strains (re-)analysed by EURL with biochemical galleries, slide agglutination, 3 PCRs, MALDI-TOFF, MLVA and WGS.





Interlaboratory study (ILS) ISO/TS 6579-4 - II

- › Feb-March 2022: Call for participants among NRLs-*Salmonella*, members of ISO/TC34/SC9 and ISO/TC34/SC9-WG10.

Number of registrations		
PCR method 1	PCR method 2	PCR method 3
probe-based multiplex real-time PCR	agarose gel-based multiplex PCR	agarose gel-based single target PCR
30	21	17

- › 6 April 2022: informing potential participants on planning ILS.
- › 26 April 2022: sending information and documents to participants ILS.
- › 16 May – 1 July 2022: Performance ILS.





ISO-AHG Harmonisation of ISO/CEN standards

- › 'Internal' ISO/CEN Guidance document to help convenors and project leaders ISO/TC34/SC9 and CEN/TC463 with the drafting of ISO/CEN documents.
- › To harmonise the content and layout of standards for microbiology of the food chain.
- › Dynamic document and will need to be updated to any new agreements made in ISO and CEN.
- › Edition 1 was published in 2018; ED2 in 2020.
- › Draft ED3 is sent to SC9 members for comments 15 April - 27 May 2022.





ISO-WG3 Method validation - AHG validation status ISO's

- › For verification of methods following EN ISO 16140-3:2021 (*'Verification of reference and validated alternative methods implemented in a single laboratory'*) the (EN ISO) reference methods have to be validated (transition period until 31-12-2027).
- › AHG made inventory on presence of performance characteristics in EN ISO documents of microbiology of the Food chain.
- › Table prepared of methods needing (additional) validation studies, in order of importance (end 2021).
- › Consultation among ISO/TC34/SC9 members to identify volunteers to organize and/or participate in validation studies on standardized methods listed (early 2022).





ISO-WG3 Method validation – Amd.1 ISO 16140-2

- › Development of Amd. 1 of EN ISO 16140-2:2016 (*'Protocol for the validation of alternative (proprietary) methods against a reference method'*) started in 2020.
- › Amd.1 will be entitled: 'Revision of the qualitative method comparison study data evaluation, revision of RLOD calculations in the interlaboratory study, revision of the calculation and interpretation of the relative trueness study, and inclusion of commercial sterility testing protocol of UHT milk'
- › CD voting took place in September-November 2021.





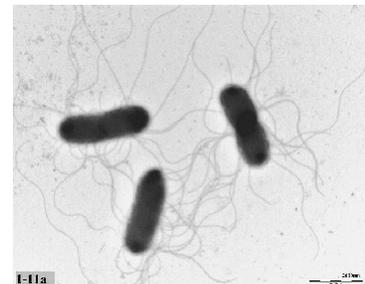
ISO-WG3 Method validation – Amd.1 ISO 16140-4

- › Development of Amd. 1 of EN ISO 16140-4:2020 (*'Protocol for method validation in a single laboratory'*) started in fall 2020.
- › Amd.1 will be entitled: 'Validation of a larger test portion size for qualitative methods'
- › CD voting took place in October-December 2021.
- › Approach in document is to compare LOD of original test portion size to LOD of larger test portion size.
- › Parallel development of Amd.1 of ISO 6887-1 (*'Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions'*), as this document contains information for verification of pooling/compositing of samples.



ISO-WG3 Method validation – Development ISO 16140-7

- › Development of ISO 16140-7 started in 2020.
- › Title: 'Protocol for the validation of identification methods of microorganisms'
- › CD voting took place in February-April 2022.
- › Scope: 'This document specifies the general principle and the technical protocol for the validation of identification methods of microorganisms for microbiology in the food chain. As there is no reference method, this document provides a protocol to evaluate the performance characteristics and validate the method workflow using well-defined strains.'
- › For use of the identification method as e.g. alternative confirmation in a reference method, validation shall be performed following ISO 16140-6.





ISO-WG3 Method validation – Revision ISO 17468

- › Revision of ISO 17468 started in 2020.
- › Title: ‘Technical requirements and guidance on establishment or revision of a standardized reference method’
- › CD voting took place in October-November 2021.
- › Revision will include:
 - Information of EN ISO 16140-4 (in-house validation), EN ISO 16140-6 (validation of confirmation and typing methods) and EN ISO 11133 (performance testing of culture media);
 - Explanation of impact of minor and major changes of a revised ISO doc;
 - Extension of content for situations where it is not possible to compare a new ISO method with a former reference method.



ISO-WG3 Method validation – WG3-WG2 sub-group

- › Joint sub-group of WG 2 (Statistics) and WG 3 (Method validation) to: 'Review evaluation/validation protocols for ISO standards'
- › Sub-group was raised in 2020.
- › The sub-group will advise working groups with drafting of the protocols for performing validation studies.
- › For example: the sub-group gave advise to the protocol of the interlaboratory study (ILS) for validation of ISO/TS 6579-4 (Identification of monophasic *Salmonella* Typhimurium).





ISO-JWG5 development concerning ISO 11133 - I

- › Title: *'Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media'*
- › ISO 11133 was published in 2014.
- › Amd.1 'Corrections and explanations' published in 2018.
- › Amd.2 'Performance testing of confirmation media and reagents' published in 2020.
- › Table with control strains for performance testing of culture media and reagents from published standards from food, feed and water microbiology published at website ISO/TC34/SC9:
<https://committee.iso.org/home/tc34sc9>

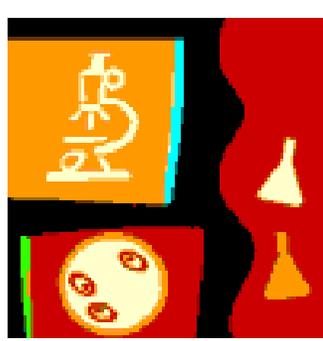




ISO-JWG5 development concerning ISO 11133 - II

- › WG will test a protocol to compare productivity and selectivity testing for different volumes of liquid media (e.g. 10 ml and 250 ml).
- › Revision of ISO 11133 will start as soon as a co-project leader from ISO/TC147/SC4 ('water microbiology') is nominated.
- › Since 2014 new or revised standards have been published containing (slightly) different specifications and/or different control strains for performance testing than ISO 11133.
- › ISO/TC34/SC9 indicates: 'Any information on performance testing of culture media and reagents described in new or revised standards published since 2014 takes precedence over ISO 11133:2014.'





ISO-WG7 Revision of ISO 7218

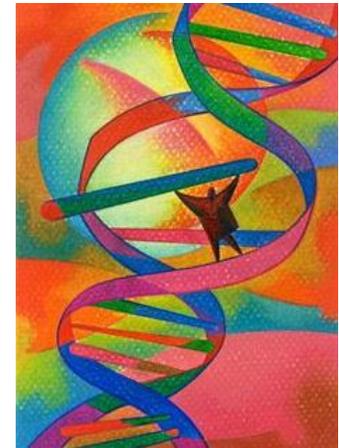
- › Revision of ISO 7218 started in 2019.
- › Title: 'Microbiology of the food chain — General requirements and guidance for microbiological examinations'
- › CD voting took place in October 2020 – January 2021.
- › Result CD voting positive, but with approx. 50 pages of comments.
- › DIS voting started on 9 May 2022, until 1 August 2022.





ISO-WG25 Development of ISO 23418

- › Development ISO 23418 started in 2019.
- › Title: 'Microbiology of the Food Chain — Whole genome sequencing for typing and genomic characterization of foodborne bacteria — General requirements and guidance'
- › Dis voting took place in September - December 2020.
- › Draft FDIS voting: September - November 2021.
- › FDIS voting: February – April 2022.
- › Publication by the end of 2022 (?)





Any questions?

