



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: International Standardisation Organisation / Technical Committee 34 on Food products / Subcommittee 9 - Microbiology of the food chain
- CEN/TC463: European Committee for Standardisation / Technical Committee 463: Microbiology of the food chain

Next annual meeting: 26-30 June 2023, Stockholm, Sweden

***Salmonella*** activities in:

ISO/TC34/SC9 – WG9 'Detection of *Salmonella*'

ISO/TC34/SC9 – WG10 'Typing of *Salmonella*'



## WG10 Typing of *Salmonella*

Development of **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)'.

Describes 3 different PCR methods, 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.



# WG10 Development of ISO/TS 6579-4

- › Preparatory work performed in CEN/TC463-WG1 (2015-2020), including method(s) evaluation study.
- › In 2020 activity moved to ISO/TC34/SC9 with vote for New Work Item Proposal (NWIP) & call for experts of WG10; Nov 2020: 1<sup>st</sup> meeting WG10.
- › Spring 2021: Voting round for draft ISO/TS CD 6579-4 among members of ISO/TC34/SC9, CEN/TC463 and NRLs-*Salmonella*. Outcome: positive with comments. → preparation 1<sup>st</sup> draft ISO/DTS 6579-4.
- › 2022: organisation ILS; summarizing performance characteristics of method evaluation and of ILS in 4<sup>th</sup> draft ISO/DTS 6579-4.
- › 2022/2023: commenting round in WG 10 of 4<sup>th</sup> and 5<sup>th</sup> draft ISO/DTS 6579-4.
- › May 2023: 6<sup>th</sup> draft ISO/DTS 6579-4 will be sent to members of ISO/TC34/SC9 and CEN/TC463 and to NRLs-*Salmonella* for comments.



# Validation of ISO/TS 6579-4

Validation performed in accordance with **ISO/DIS 17468:2022** ('Microbiology of the food chain — Technical requirements and guidance on establishment or revision of a standardized reference method').

For confirmation and typing methods validation study is based upon **EN ISO 16140-6:2019** ('Microbiology of the food chain – Method validation - Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures').

Validation study performed to determine **performance characteristics**; existed of two parts:

- Method(s) evaluation study
- Interlaboratory study



## Performance characteristics ISO/TS 6579-4

- › Performance characteristics of confirmation/typing methods are **Inclusivity and Exclusivity**.
- › Definitions according to EN ISO 16140-1:
  - Inclusivity study: study involving pure target strains to be detected or enumerated by the method.
  - Exclusivity study: study involving pure non-target strains, which can be potentially cross-reactive, but are not expected to be detected or enumerated by the method.
- › Performance characteristics obtained from method(s) evaluation study and ILS summarized in Annex E of (draft) ISO/TS 6579-4.



## Method(s) evaluation study

- › In 2018 and 2019, the 3 PCR protocols were tested in a method(s) evaluation study in 2 different laboratories (DE and NL).
- › For inclusivity and exclusivity testing, results of 'new' method have to be compared to a 'reference method'. For *Salmonella* strains slide agglutination was considered as reference method.
- › 172 different strains were tested:
  - 53 monophasic *Salmonella* Typhimurium strains;
  - 46 biphasic *Salmonella* Typhimurium strains;
  - 46 strains of other *Salmonella* serovars (not monophasic *Salmonella* Typhimurium, not biphasic *Salmonella* Typhimurium);
  - 27 strains of other *Enterobacteriaceae* (not *Salmonella*).





## Performance characteristics method(s) evaluation study

- › It depends on intended purpose of the strain identification (see scope) if only monophasic *Salmonella* Typhimurium (1,4,[5],12:i:-) is considered as target strain or if (biphasic) *Salmonella* Typhimurium (1,4,[5],12:i:1,2) is considered as target strain as well.
- › EU regulatory limits in poultry meat are the same for *Salmonella* Typhimurium (STm) and its monophasic variant → important to know if strain under analysis is monophasic variant of *Salmonella* Typhimurium (mSTm) and not monophasic variant of another *Salmonella* non-Typhimurium (with same antigenic formula). In this case mSTm and STm are both considered as target strains (Table E.1).
- › If aim is to identify if strain is either mSTm or STm, then STm considered as non-target strain (Table E.2).

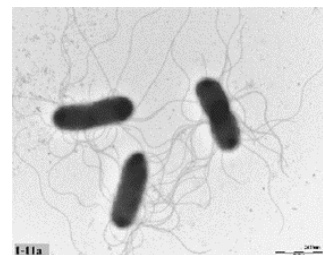






Table E.1 Inclusivity and exclusivity results of the method evaluation study performed in two laboratories with the three PCR protocols; considering both monophasic *Salmonella* Typhimurium (mSTm) and (biphasic) *Salmonella* Typhimurium (STm) as target strains and other *Salmonella* serovars and other *Enterobacteriaceae* as non-target strains

<b>Method</b>	<b>Performance characteristic</b>	<b>Number of different strains</b>	<b>Total number of results</b>	<b>Inclusivity agreement</b>	<b>Inclusivity deviation</b>	<b>Exclusivity agreement</b>	<b>Exclusivity deviation</b>
Multiplex real-time PCR (Annex B)	Inclusivity (mSTm + STm)	99	198	198	0	Not applicable	Not applicable
	Exclusivity	73	146	Not applicable	Not applicable	146	0
Gel-based multiplex PCR (Annex C)	Inclusivity (mSTm + STm)	99	198	198	0	Not applicable	Not applicable
	Exclusivity	73	146	Not applicable	Not applicable	146	0
Gel-based single target PCR (Annex D)	Inclusivity (mSTm + STm)	99	198	198	0	Not applicable	Not applicable
	Exclusivity	73	146	Not applicable	Not applicable	146	0



Table E.2 Inclusivity and exclusivity results of the method evaluation study performed in two laboratories with the three PCR protocols; considering monophasic *Salmonella* Typhimurium (mSTm) as target strain and (biphasic) *Salmonella* Typhimurium (STm), other *Salmonella* serovars and other *Enterobacteriaceae* as non-target strains

Method	Performance characteristic	Number of different strains	Total number of results	Inclusivity agreement	Inclusivity deviation	Exclusivity agreement	Exclusivity deviation
Multiplex real-time PCR (Annex B)	Inclusivity (mSTm)	53	106	106	0	NA	NA
	Exclusivity (STm)	46	92	NA	NA	82	10 <sup>a</sup>
	Exclusivity (other <i>S. serovars</i> and <i>Enteroc's</i> )	73	146	NA	NA	146	0
Gel-based multiplex PCR (Annex C)	Inclusivity (mSTm)	53	106	76	30 <sup>b</sup>	NA	NA
	Exclusivity (STm)	46	92	NA	NA	88	4 <sup>c</sup>
	Exclusivity (other <i>S. serovars</i> and <i>Enteroc's</i> )	73	146	NA	NA	146	0
Gel-based single target PCR (Annex D)	Inclusivity (mSTm)	53	106	106	0	NA	NA
	Exclusivity (STm)	46	92	NA	NA	86	6 <sup>d</sup>
	Exclusivity (other <i>S. serovars</i> and <i>Enteroc's</i> )	73	146	NA	NA	146	0

<sup>a</sup>: 5 different strains were tested as monophasic *Salmonella* Typhimurium instead of (biphasic) *Salmonella* Typhimurium by both laboratories.

<sup>b</sup>: 15 different strains were tested as (biphasic) *Salmonella* Typhimurium instead of monophasic *Salmonella* Typhimurium by both laboratories.

<sup>c</sup>: 2 different strains were tested as monophasic *Salmonella* Typhimurium instead of (biphasic) *Salmonella* Typhimurium by both laboratories.

<sup>d</sup>: 1 strain was tested as mSTm instead of STm by both labs and 4 different strains were tested as mSTm by 1 lab and as STm by the other lab.



Table E.3 Details of the interlaboratory study of the three PCR protocols.

<b>Details of the interlaboratory study</b>				
<b>Method</b>	<b>Number of participating collaborators</b>	<b>Number of samples per collaborator</b>	<b>Number of collaborators retained after evaluation of data</b>	<b>Number of samples retained after evaluation of the data</b>
Multiplex real-time PCR (Annex B)	26	25	20	500
Gel-based multiplex PCR (Annex C)	18	25	17	425
Gel-based single target PCR (Annex D)	13	25	12	280 <sup>a</sup>

<sup>a</sup>20 missing values



Table E.4 Inclusivity and exclusivity results of the interlaboratory study of the three PCR protocols; considering monophasic *Salmonella* Typhimurium as target strain and (biphasic) *Salmonella* Typhimurium, other *Salmonella* serovars and other *Enterobacteriaceae* as non-target strains

Method	Performance characteristic	Number of different strains	Total number of results	Inclusivity agreement	Inclusivity deviation <sup>a</sup>	Exclusivity agreement	Exclusivity deviation <sup>a</sup>
Multiplex real-time PCR (Annex B)	Inclusivity	16	320	319	1	NA	NA
	Exclusivity	9	180	NA	NA	179	1
Gel-based multiplex PCR (Annex C)	Inclusivity	16	272	255	17	NA	NA
	Exclusivity	9	153	NA	NA	152	1
Gel-based single target PCR (Annex D)	Inclusivity	16	177 <sup>b</sup>	167	10	NA	NA
	Exclusivity	9	103 <sup>c</sup>	NA	NA	102	1

<sup>a</sup>: More information about the inclusivity deviations and exclusivity deviations is given in the last three paragraphs in clause E.2

<sup>b</sup> 15 missing values

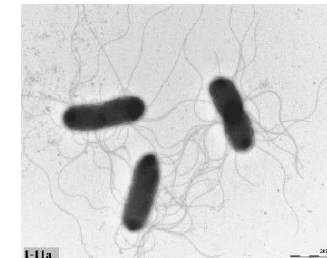
<sup>c</sup> 5 missing values



# WG10 Development of ISO/TS 6579-4 - Planning

- › Considering possible comments from the CEN/TC 463- and ISO/TC 34/SC 9- members and from NRLs-*Salmonella* on 6<sup>th</sup> draft ISO/DTS 6579-4 and incorporate those where needed.
- › Submission of ISO/DTS 6579-4 to ISO/Central Secretariat in September 2023 for the start of the ISO/DTS ballot (last voting step) for 6 weeks – 3 months.
- › Considering possible comments to ISO/DTS 6579-4 and incorporate those where needed. Preparation of final ISO/TS 6579-4 by the end of 2023/ early 2024.
- › Publication of ISO/TS 6579-4 in 2024.





## WG9 Detection of *Salmonella*

**EN ISO 6579-1:2017/Amd.1:2020** 'Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp. – Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSR/V and SC'

- › Jan-June 2022: 5 years systematic review of ISO 6579-1:2017. Result of voting: 25 members confirmed, 3 voted revision, 0 voted withdrawal, 14 abstained. In total 3 pages comments.
- › June 2022: agreed at annual meeting ISO/TC34/SC9 and CEN/TC463 to re-activate WG9.
- › August-September 2022: call for members and convenor of WG9; (re)appointment Kirsten Mooijman as convenor of WG9 and NEN as secretariat.



## Aims WG9 Detection of *Salmonella*

1. To complete the performance characteristics of ISO 6579-1 ('Detection of *Salmonella*) for all relevant product categories (broad range of food, animal feed, environmental samples and samples from the primary production stage).
2. To consider the comments to ISO 6579-1:2017 from the systematic review of 2022.

Aim 1 is needed, as 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). If reference method is not validated for relevant product categories (e.g. animal feed, or not for a broad range of food), the user has to perform a (in-house) validation instead of verification.



# Performance characteristics published in EN ISO 6579-1:2017

Category	Item	Test portion size (g)	Specificity (%)	Sensitivity (%)		LOD <sub>50</sub> (95% ci) in cfu/test portion
				Low level	High level	
Dairy products	Fresh cheese curd	25	100	74,3	83,8	5,7 (4,0-8,1)
Egg products	Egg powder	25	100	98,1	99,0	6,0 (4,7-7,7)
Raw poultry meat	Raw poultry meat	25	100	98	100	2,2 (1,5-3,2)
Primary production stage (PPS)	Chicken faeces	10	100	67,4 & 96,8	100	2,5 (2,1-3,0)
PPS	Pig faeces	25	99,2	88,5 & 91,5	97,7 & 98,5	3,2 (2,8-3,8)
PPS	Boot socks + 10 g environmental material	Boot socks	99,6	94,7	98,1	3,8 (3,2-4,4)





## Missing performance characteristics in EN ISO 6579-1:2017

- › 2 Additional food categories (to make sure that the standard is validated for a broad range of food);
- › Animal feed (like cereals, flours, bone meal, soybean meal, fish, meat);
- › Environmental samples from the food or feed production (like swabs, dusts, process water);
- › Larger test portion sizes (>25 g);
- › LOD<sub>50</sub> values for use of MSR/V agar as (second) selective enrichment medium for analysing food/feed/environmental samples.



→ Discussed at 1<sup>st</sup> (online) meeting of WG9 in March 2023 how to generate missing performance characteristics.

# How to generate missing performance characteristics?

	<b>Pros</b>	<b>Cons</b>
Organisation of Interlaboratory studies for each missing category	<ul style="list-style-type: none"> <li>- Organisation in own hands.</li> <li>- Own choice of matrices.</li> <li>- Choice of <i>Salmonella</i> serovars in own hands.</li> <li>- Contamination levels in own hands.</li> </ul>	<ul style="list-style-type: none"> <li>- Difficult to find organiser and collaborators.</li> <li>- Labor intensive and expensive for organiser.</li> <li>- Labor intensive for collaborators.</li> <li>- Takes much time to organize 4 ILS's.</li> </ul>
Use data of validation studies (of ILS) of AFNOR, MicroVal, AOAC (if performed in accordance with ISO 16140-2)	<ul style="list-style-type: none"> <li>- No need for organization of ILS.</li> <li>- Data available on short notice.</li> </ul>	<ul style="list-style-type: none"> <li>- Validation data (ILS) for matrix of choice may not be available.</li> <li>- No influence on choice of <i>Salmonella</i> serovar.</li> <li>- Contamination levels may not always comply with demands.</li> </ul>
Use data of EURL- <i>Salmonella</i> PTs (historical data and new PTs), or of NRL- <i>Salmonella</i> PTs if organized for >1 country	<ul style="list-style-type: none"> <li>- Choice of matrix (partly) in own hands.</li> <li>- Large group of (experienced) participants (NRLs-<i>Salmonella</i>) from all EU MS.</li> <li>- Total number of data may be sufficient although number of samples per level and per lab may not be in accordance with EN ISO 16140-2.</li> </ul>	<ul style="list-style-type: none"> <li>- Aim PT differs from aim ILS.</li> <li>- Contamination low level samples not sufficiently low (no fractional recovery).</li> <li>- Number of samples per level and per lab not in accordance with EN ISO 16140-2.</li> </ul>



## Protocol for ILS of qualitative methods

Protocol needs to be in accordance with ISO/DIS 17468:2022, EN ISO 16140-2:2016 (clause 5.2.2) and Draft ISO 16140-2:2016/DAmD.1:2023:

- At least 10 valid datasets from at least 10 participants;
- Participants from at least 5 different organisations;
- Participants from at least 2 different countries;
- Maximum of 3 datasets/organisation;
- 8 samples per level and item to be tested:
  - 8x negative
  - 8x low level to obtain fractional recovery (approx. 1 cfu/sample)
  - 8x high level (approx. 5-10x low level)
- In total at least 240 results needed (80 results per level).

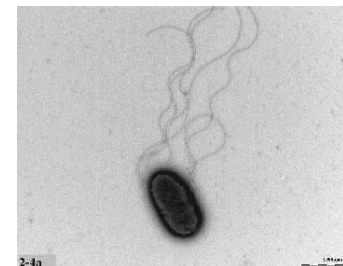




# First meeting WG9 Detection of *Salmonella*

Decisions taken at online meeting of 28 March 2023:

- › Agreed about replies to the systematic review of 2022;
- › Agreed to start with literature review (especially Afnor and MicroVal validations) of validation studies. For this an Excel template was sent to WG9 members in April 2023.
- › To contact some organisations (e.g. Nestlé) for possible validation data of larger test portions.
- › For now, to confirm ISO 6579-1:2017 (and its amendment). To start its revision once the additional validation data for ISO 6579-1 are obtained. So that Amd.1 and validation data are incorporated into one document.
- › To plan a 2<sup>nd</sup> WG9 meeting in the 2<sup>nd</sup> half of 2023.





## ISO-AHG A Harmonisation of ISO/CEN standards

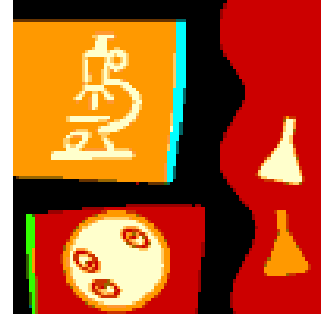
- › 'Internal' ISO/CEN Guidance document to help convenors and project leaders ISO/TC34/SC9 and CEN/TC463 with the drafting of ISO/CEN documents.
- › To harmonise the content and layout of standards for microbiology of the food chain.
- › Dynamic document and will need to be updated to any new agreements made in ISO and CEN.
- › Edition 1 was published in 2018; ED2 in 2020.
- › Edition 3 was published in August 2022.





## ISO-AHG E General terms and definitions

- › ISO-AHG A identified that definitions of some general terms are missing, e.g. Food chain, products intended for human consumption, products for feeding animals, primary production stage.
- › AHG E was raised by the end of 2022.
- › During 2 meetings (January and April 2023) draft definitions were discussed.
- › 19/4-30/5 2023: call for comments (in SC9) to proposed definitions for inclusion in (draft) ISO 7218.



## 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'
- › Committee Draft (CD) voting: Oct 2020- Jan 2021; positive with 50 pages comments.
- › Draft International Standard (DIS) voting: May-August 2022; positive with 92 pages comments.
- › Final Draft International Standard (FDIS) voting (last voting step, only for editorial comments) expected in 2023.



## ISO-WG3 Method validation – AHG C 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 started in 2020 and 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).
- › Drafting guidelines for ISO/CEN WGs for generation and use of LOD<sub>50</sub> data from validation studies of e.g. MicroVal and AFNOR.







## 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 is 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: September-November 2021.
- › DIS voting expected in Summer 2023.





## ISO-WG3 Method validation – Revision ISO 16140-1 & -2

- › Future revision of EN ISO 16140-1:2016 (*'Method validation – Vocabulary'*)
- › Future revision of EN ISO 16140-2:2016 (*'Protocol for the validation of alternative (proprietary) methods against a reference method'*)
- › Parallel timeline for both parts
- › Official revision will start after publication of ISO 16140-2/Amd1 (2024)
- › Preparatory work started in April 2023





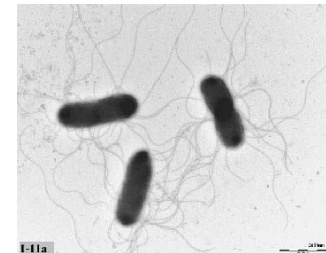
## ISO-WG3 Method validation – Amd.1 ISO 16140-4

- › Development of Amd. 1 of EN ISO 16140-4:2020 (*'Protocol for method validation in a single laboratory'*) started in fall 2020.
- › Amd.1 is entitled: 'Validation of a larger test portion size for qualitative methods'
- › Approach in document is to compare  $LOD_{50}$  of original test portion size to  $LOD_{50}$  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.
- › CD voting: October-December 2021.
- › DIS voting expected in Summer 2023.



## 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'
- › 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.'
- › Remark: for use of the identification method as e.g. alternative confirmation in a reference method, validation shall be performed following ISO 16140-6.
- › CD voting: February-April 2022.
- › DIS voting: May-July 2023.





## 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'
- › Revision includes:
  - Inclusion of information on 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.
- › CD voting: October-November 2021.
- › DIS voting: September-December 2022.
- › FDIS voting expected second half 2023.





## 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 advises working groups with drafting of the protocols for performing validation studies.
- › For example: the sub-group advised on the protocol of the interlaboratory study (ILS) for validation of ISO/TS 6579-4 (Identification of monophasic *Salmonella* Typhimurium).





## ISO-JWG5 revision ISO 11133

- › 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>
- › Revision started with commenting round on ISO/WD 11133 in March 2023 (until mid-April 2023).



Any questions?

