

Update ISO and CEN activities

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EURL-*Campylobacter* workshop
2023



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Current relevant ISO work

- ISO/TC 34/SC 9 Food products – Microbiology
 - **WG 2 ‘Statistics’**: Will combine results from the EURL-study “matrix uncertainty” and a study in France to a common database on SC9 website and write a guide on how to use the data.
 - **WG 3 ‘Method validation’**: see next slides
 - **WG 7 ‘General requirements and guidance for microbiological examinations’**:
 - Revision of ISO 7218 (current version from 2007, Amd 2013), FDIS autumn 2023
 - **WG8 ‘Preparation of test samples, initial suspensions and decimal dilutions’**
 - Revision will be started soon of ISO 6887 part 1 (General rules for the preparation of the initial suspension and decimal dilutions) and 4 (Specific rules for the preparation of miscellaneous products).
 - **WG35 ‘Sampling techniques’**:
 - Revision will be started soon of ISO 17728 (‘Microbiology of the food chain - Sampling techniques for microbiological analysis of food and feed samples’).

ISO/TC 34/SC 9/WG 3: Method validation

- **ISO 17464** ‘Technical requirements and guidance on the establishment or revision of a standardized reference method’ will be published soon.
- ISO 16140 series
 - **ISO 16140-2:2016/draft DAmd.1** ‘Protocol for the validation of alternative (proprietary) methods against a reference method — Amendment 1 is under development (Enquiry stage). Includes e.g.: RLOD calculations in the ILS and calculation and interpretation of the relative trueness study.
 - **ISO 16140-1** (terminology) and -2 will begin full revision after finishing of Amd1.
 - **ISO 16140-4:2020/DAmd.1** ‘Protocol for method validation in a single laboratory — Amendment 1: Protocol for validation of larger test portion size in qualitative methods’. (Enquiry stage).
 - **ISO 16140-7** ‘Protocol for the validation of methods for the identification of microorganisms’’: FDIS to be expected autumn 2023.
 - Project-group on viruses and parasites (**16140-8**), and one for bacterial toxins.

EN ISO 16140 series in Reg. (EC) No. 2073/2005

- The intent by ISO/TC 34/SC9 is to introduce ISO 16140-3, 4 and 6 into Regulation (EC) No. 2073/2005.
- A proposal for amended text in Article 5 'Specific rules for testing and sampling' has been circulated to SC9 members 2022 and sent to DG SANTE.
- This text proposes to:
 - Add EN ISO 16140-3 (verification) as a requirement for new verifications (those done before publication of 16140-3 ok).
 - Add information about of EN ISO 16140-4 (validation in single laboratory) and -6 (confirmation methods).
- DG SANTE commented that the text needs to be simplified and some technical details changed before it can be proposed to the MS.
- A second version of the text is expected to be drafted by October 2023.

CEN/TC 463/WG3 Campylobacter

- **Publication of Amendment 1 to EN ISO 10272-1 and 2**
 - **AMENDMENT 1 to ISO 10272:2017¹ -1:** Inclusion of methods for molecular confirmation and identification of thermotolerant *Campylobacter* spp. the use of growth supplement in Preston broth and changes in the performance testing of culture media”
 - **AMENDMENT 1 to ISO 10272:2017¹ -2:** Inclusion of methods for molecular confirmation and identification of thermotolerant *Campylobacter* spp. and changes in the performance testing of culture media”
 - Publication January 2023.
- FDIS opened 2022-09-02, closed 2022-10-28 2022. 100% approval at CEN and ISO level on both amendments. The amendment generated 24 comments for part 1 and 22 comments for part 2. The comments were mainly of editorial and general character, and mostly accepted before publication.

¹EN ISO 17025:2017 Microbiology of the food chain — Horizontal method for detection and enumeration of *Campylobacter* spp.

Amendment 1 to EN ISO 10272-1 and 2

- The main changes in amendments;
 - The addition of 3 informative annexes:
 - **Annex D:** A PCR method for confirmation of thermotolerant *Campylobacter* spp. Real-time PCR targeting *C. jejuni*, *C. coli* and *C. lari*.
 - **Annex E:** Two PCR methods for confirmation and identification of thermotolerant *Campylobacter* spp. Gel-based targeting *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis* and real-time PCR targeting *C. jejuni*, *C. coli* and *C. lari*.
 - **Annex F:** The results of an interlaboratory study (ILS) for validation of the PCR methods.
 - Updates of table B1 “Performance testing of culture media for *Campylobacter*”.
 - Update of description of indoxyl acetate test.
 - For part 1: Update of composition of Preston broth (inclusion of growth supplement)
 - Supporting data are available at: <https://standards.iso.org/iso/10272/-1/ed-1/en/amd/1/>

Additional publication

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Validation of PCR methods for confirmation and species identification of thermotolerant *Campylobacter* as part of EN ISO 10272 - Microbiology of the food chain - Horizontal method for detection and enumeration of *Campylobacter* spp.

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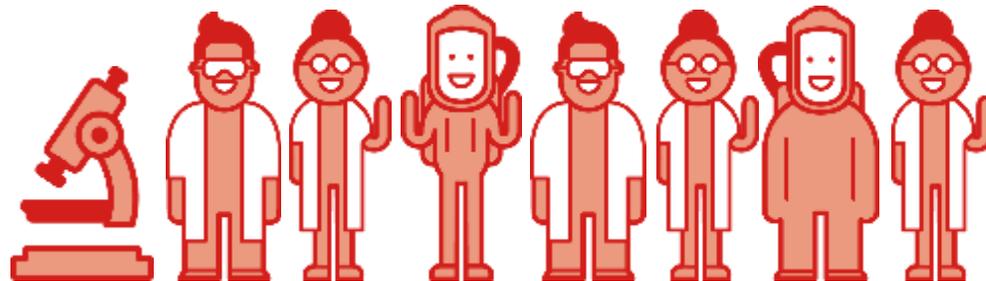
Next steps in CEN/TC 463/WG 3 *Campylobacter*

1. Functionality of Preston broth

2. Validation of ISO 10272

- Validation of larger test portion size?
 - Validation of larger test portion sizes are valid for smaller test portion sizes but not the other way around.
- Validation of an additional food matrix to claim validation for 'broad range of foods'

Next meeting 7th of December 2023



Functionality of Preston broth

- The composition of Preston broth was changed in Amd1 with the addition of growth supplement.
- Collect information from NRLs-*Campylobacter* about the overall application and functioning of the three different procedures (and outcome after Amd1): issues with performance testing, the use of alternative broths, and about the prevalence of β -lactam resistant bacteria. **Information collected through the Pre-workshop survey.**
- The EURL-*Campylobacter* will perform a literature review to screen for possible improvements/replacements of Preston broth. If interesting candidates are found, test them at EURL 2024 and maybe include in PT 2025?
- If there is data to support the replacement of Preston broth we need to move to a full revision of ISO 10272

Validation of larger test portion size?

- The WG asked laboratories reported to use 25g for more information (purpose, for which matrices and if available LOD₅₀ or eLOD₅₀ data for 25g/10g):
 - Data collected in April-May 2023.
 - **Purposes to use 25g:** Customer demands, long-standing laboratory routines, national regulation in Italy concerning raw milk, expectation of improved detection in samples with low contamination of *Campylobacter*.
 - **For which matrices are 25g used:** More commonly used for specific matrices than for all matrices analysed in the laboratory for *Campylobacter*.
 - **LOD₅₀ or eLOD₅₀.** Only two datasets were obtained:
 - eLOD₅₀ turkey strips and pluck lettuce: no difference between 10g or 25g
 - LOD₅₀ (same matrices as in validation study): next slide

In-house comparison of LOD₅₀ for 10g vs 25g of same matrices as validated in ISO 10272

- Sample size does not seem to have major impact on the LOD₅₀ for *Campylobacter* in chicken skin, raw milk, frozen spinach and minced meat¹.
- Follow-up study: same matrices were tested with all five strains and procedures. LOD₅₀ strongly dependent on strain, procedure and food matrix².
- Sample size likely has a smaller impact on LOD₅₀ than strain, procedure and food matrix used.

Sample size tests	Chicken skin		Raw milk		Frozen spinach		Minced meat	
	Detection method	Preston broth/mCCDA		Preston broth/mCCDA		Bolton broth/mCCDA		Bolton broth/mCCDA
Inoculation strain	<i>C. coli</i> WDCM 00004		<i>C. jejuni</i> WDCM 00156		<i>C. jejuni</i> WDCM 00005		<i>C. coli</i> WDCM 00072	
Level of inoculum	Low	High	Low	High	Low	High	Low	High
Number of samples tested	20	8	20	8	20	8	20	8
Number of positive samples	16	8	11	7	16	8	7	5
Sample size in g or ml	10	10	10	10	10	10	10	10
Level of inoculum (cfu/sample)	0,9	3,6	4,8	19,2	0,1	0,5	0,5	2
LOD ₅₀ (95% confidence interval) in cfu/sample	0,4 (0,0 - 0,7)		4,8 (0,3 - 8,3)		0,1 (0,0 - 0,1)		1,0 (0,1 - 1,9)	

10g →

Sample size tests	Chicken skin		Raw milk		Frozen spinach		Minced meat	
	Detection method	Preston broth/mCCDA		Preston broth/mCCDA		Bolton broth/mCCDA		Bolton broth/mCCDA
Inoculation strain	<i>C. coli</i> WDCM 00004		<i>C. jejuni</i> WDCM 00156		<i>C. jejuni</i> WDCM 00005		<i>C. coli</i> WDCM 00072	
Level of inoculum	Low	High	Low	High	Low	High	Low	High
Number of samples tested	20	8	20	8	20	8	20	8
Number of positive samples	10	8	16	8	12	8	2	5
Sample size in g or ml	25	25	25	25	25	25	25	25
Level of inoculum (cfu/sample)	0,9	3,6	4,8	19,2	0,1	0,5	0,5	2
LOD ₅₀ (95% confidence interval) in cfu/sample	0,8 (0,0 - 1,3)		2,1 (0,1 - 3,5)		0,1 (0,0 - 0,2)		2,0 (0,0 - 4,2)	

25g →

1 Poster CHRO conference 2015: Wilma Jacobs-Reitsma, Wendy van Overbeek, Wilma Hazeleger, Ida Jongenburger. Impact of sample size on Limit of Detection LOD50 to detect *Campylobacter* in foods.

2 Frontiers in Microbiology: Wilma C. Hazeleger, Wilma F. Jacobs-Reitsma and Heidi M. W. Den Besten. 2022. Level of Detection (LOD50) of *Campylobacter* Is Strongly Dependent on Strain, Enrichment Broth, and Food Matrix.

Current plan on test portion size

- Autumn 2023: call for more validation data in ISO/ TC 34/SC9/ for for 10 g and larger test portion sizes using ISO 10272-1:2017 (or Amd1)
- If a larger test portion size clearly improves the method over 10 g, WG3 have decided to redo the validation study for a larger test portion size. If not, we will at least wait until the next full revision of the method.

Validation of an additional food matrix to claim validation for 'broad range of foods'

- At this stage, it is not possible to set a time-frame for validation of a fifth food category since it depends on if the method will be revised or not.
- The WG has proposed to validate the category "fresh produce and fruits".
- The EURL-*Campylobacter* has agreed to propose to DG SANTE to organise the validation study as part of a PT for enumeration and as an ILS for detection. If the method is not revised, this would likely be in 2026.

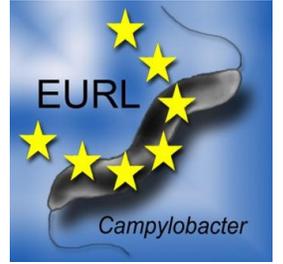


Additional validation to claim 'broad range of foods' – validation of different procedures

- The intention in ISO 10272-1 is that the user should decide when to use the different procedures based on expected level of background flora and level of *Campylobacter* and of β -lactam-resistant bacteria present in the matrix.
- ISO 10272-1 says 'If little information is available concerning the best method for the particular type of sample to be tested, then use detection procedure C, in parallel with detection procedure(s) A and/or B.'
- The application of ISO 10272-1 in practice does not always follow the examples given in the standard.
- We have received a confirmation that in order to claim a 'broad range of foods' validation – all procedures does NOT need to be validated for five food categories each – Five food categories in total is what is required also of a horizontal method. Hope this will not cause an issue with accreditations?



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Thank you for your attention!

Questions?

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