EURL-*CAMPYLOBACTER*

REPORT

PROFICIENCY TEST NUMBER 35

Detection and species identification of *Campylobacter* spp.

Publication history

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Contents

Abbreviations

BPW	buffered peptone water
C.	*Campylobacter*
cfu	colony forming units
EU	European Union
EURL	European Union reference laboratory
ISO	International Organization for Standardization
LOD_{50}	level of detection for which 50 % of tests give a positive result
log_{10}	logarithm to base 10 (common logarithm)
MALDI-TOF MS	matrix-assisted laser desorption ionization–time of flight mass spectrometry
mCCD	modified charcoal cefoperazone deoxycholate
MS	Member State (of the European Union)
MS-NRL	Member State national reference laboratory
No.	number
NRL	national reference laboratory (in this report used for all participating laboratories, also in non-EU countries)
PCR	polymerase chain reaction
PT	proficiency test
SD	standard deviation
spp.	species

Summary of proficiency test number 35, 2023

The EU reference laboratory for *Campylobacter* organised proficiency test (PT) number 35 on detection and species identification of *Campylobacter* in March 2023. The PT contained 18 boot sock samples simulated to have been taken from chicken houses and were to be mixed with vials with or without freeze-dried *Campylobacter*. The objective was to assess the performance of the national reference laboratories (NRLs) to detect and identify *Campylobacter* species in boot sock samples.

Participation in PT 35 was mandatory for at least one NRL per Member State. Thirty-three NRLs in 27 EU Member States (some Member States have more than one NRL) and in Iceland, Norway, Republic of North Macedonia, Switzerland, and United Kingdom received the PT, and 32 NRLs reported results.

Of the 32 NRLs reporting results, 31 followed the recommended method ISO 10272-1:2017 for detection of *Campylobacter* spp., and one NRL used another method. All except two NRLs performed enrichment procedure in Preston broth. Fifteen NRLs used direct plating, with one exception in addition to enrichment procedures.

A combined five-level grading scale for performance in detection was based on minimum limits for sensitivity in detection of low-level samples, sensitivity in detection of high-level samples and specificity. Of the 32 participating NRLs, twenty-five NRLs (78 %) fulfilled the criterion for excellent or good performance in detection of *Campylobacter*, and three (two Member State NRLs, MS-NRLs) scored below the acceptable limit, whereof two failed to detect any *Campylobacter* at all. One additional MS-NRL failed to report final results.

Twenty-six NRLs fulfilled the criterion for excellent or good performance in identification of *Campylobacter* spp., and three (one MS-NRL) either scored below the acceptable limit or did not present a result because of poor performance in the detection part.

In summary, the majority of the NRLs met the criteria for excellent or good performance in both detection and species identification. Three NRLs scored below the acceptable limit in detection. The two underperforming MS-NRLs and the MS-NRL that failed to report final results were offered and performed an extra PT.

Introduction

The proficiency test (PT) number 35 on detection and species identification of *Campylobacter* was organised by the European Union reference laboratory (EURL) for *Campylobacter* in March 2023. Participation in the PT was mandatory for at least one NRL in each Member State.

Thirty-three national reference laboratories (NRLs) in 27 EU Member States (some Member States have more than one NRL) and in Iceland, Norway, Republic of North Macedonia, Switzerland, and United Kingdom received the PT. The test results and operational details were reported to the EURL from 32 NRLs. Twenty-nine NRLs reported that they were accredited for detection of *Campylobacter* and 21 were also accredited for enumeration of *Campylobacter*.

The PT included detection and species identification of *Campylobacter* spp. in 18 boot sock samples simulated to have been taken from chicken houses and were to be mixed with vials with or without freeze-dried *Campylobacter* (Table 1). The objective was to assess the performance of the NRLs to detect and identify *Campylobacter* species in boot sock samples.

Table 1. Bacteria in the vials in proficiency test No. 35, 2023.

Vial No.	Bacterial species in vial	Batch No.	Level *Campylobacter* [a] (log_{10} cfu/vial & log_{10} cfu/test portion)			Level *E. coli* [b] (log_{10} cfu/vial & log_{10} cfu/test portion)		SD [c] (log_{10} cfu)
11	*Campylobacter jejuni* [d]	SVA074	4.85	2.45	(low)			0.08
12	*Escherichia coli*	SVA079				4.29	1.89	0.06
13	–							
14	*Campylobacter coli*	SVA075	4.46	2.06	(low)			0.05
15	*Campylobacter lari*	SVA080	5.78	3.38	(high)			0.08
16	*Escherichia coli*	SVA079				4.29	1.89	0.06
17	*Campylobacter lari*	SVA080	5.78	3.38	(high)			0.08
18	*Campylobacter lari*	SVA078	4.76	2.36	(low)			0.06
19	*Escherichia coli*	SVA079				4.29	1.89	0.06
20	*Campylobacter lari*	SVA078	4.76	2.36	(low)			0.06
21	*Campylobacter coli*	SVA076	5.28	2.88	(high)			0.08
22	*Campylobacter jejuni* [d]	SVA073	7.12	4.72	(high)			0.06
23	–							
24	*Campylobacter jejuni* [d]	SVA073	7.12	4.72	(high)			0.06
25	*Campylobacter coli*	SVA075	4.46	2.06	(low)			0.05
26	*Campylobacter jejuni* [d]	SVA074	4.85	2.45	(low)			0.08
27	–							
28	*Campylobacter coli*	SVA076	5.28	2.88	(high)			0.08

[a] Total quantity of *Campylobacter* in each vial and per test portion: 2 ml of diluted suspension (dilution factor 500), which was the amount added to each boot sock sample.

[b] Total quantity of *Escherichia coli* in each vial and per test portion (boot sock sample).

[c] Standard deviation (SD) of the level defined by homogeneity test of 10 vials after the production. The maximum SD allowed was 0.15 log_{10} cfu.

[d] All *Campylobacter jejuni* strains were hippurate positive.

Terms and definitions

- *Campylobacter* spp.: Thermotolerant *Campylobacter* spp., i.e. which are able to grow at 41.5 °C, foremost (but not exclusively) *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, and *Campylobacter upsaliensis*.
- Detection of *Campylobacter* spp.: Determination of the presence or absence of *Campylobacter* spp.
- Confirmation of *Campylobacter* spp.: Microorganisms suspected to be *Campylobacter* spp. are confirmed as such by biochemical tests and/or by molecular methods.
- Species identification of *Campylobacter*: Identification of thermotolerant *Campylobacter* species with biochemical tests and/or molecular methods.

Outline of the proficiency test

The PT contained 18 boot sock samples that were composed to mimic sock samples taken in a chicken house at a farm with conventional rearing (birds kept indoors) and were to be mixed with vials with or without *Campylobacter*.

The participants were instructed to reconstitute each vial in a volume of 5 ml buffered peptone water (BPW) and then make two ten-fold dilutions. From each final dilution, 2 ml were to be transferred to the stomacher bag containing the boot sock. This resulted in six samples with a low content of *Campylobacter*, six samples with a high content of *Campylobacter*, and six samples without *Campylobacter* (Table 1). The theoretical levels of contamination in the test portions of the low-level samples were estimated to be between 14 and 35 times an assumed LOD_{50} of about 8 cfu (based on unpublished data and the LOD_{50} for chicken caecum according to ISO 10272-1:2017, annex C) and in high-level samples at least 95 × LOD_{50}. The levels were higher than recommended in ISO 22117:2019 but were selected to guarantee that any instability during transport would not affect the evaluation of NRLs' performance. The final dilution factor was selected after testing all samples under simulated transport conditions (Table 2), resulting in theoretical levels 0.5 $\log_{10}$ cfu higher per test portion than with the original concentrations tested.

Preparation of the matrix

The caecal material and the litter material used as matrices in the PT were obtained from broiler producers that had not delivered any *Campylobacter*-positive flocks to slaughter for more than one year. The broilers were slaughtered at a slaughterhouse with a history of low level of *Campylobacter*-positive flocks (3.1 % during 2022). The chicken caecal material was tested for presence of *Campylobacter* spp. by direct streak onto modified charcoal cefoperazone deoxycholate (mCCD) agar and tested negative for presence of *Campylobacter*. The chicken caeca were freeze-stored until preparation of the PT.

Three days before dispatch, freeze-stored caeca were thawed, cut, placed in a stomacher bag and mixed with BPW. The mixed suspensions were pooled into three batches and further mixed with Cary Blair transport medium to a suitable consistency. For each sample, 20 ml of the thoroughly mixed suspension were added to a plastic bag with a boot sock. All samples

of a specific number were made from the same batch of caecal suspension. The remaining suspension from the three batches were pooled into a fourth batch that was used for making the *Campylobacter*-negative samples (intended for mixture with vials No. 12, 13, 16, 19, 23, and 27). A small amount of litter material was also added to each sample. The samples were stored at 4 °C over the weekend.

Production and quality control of the vials

The vials with freeze-dried bacterial cultures used in the PT were produced and tested for homogeneity and stability by the EURL in both storage and transport conditions.

To ensure that the level of instability during transport conditions did not affect the performance evaluation, each combination of vial batch and matrix was prepared and tested under various transport conditions (Table 2). The tests were performed according to ISO 10272-1:2017 (including ISO 10272-1:2017/Amd 1:2023), detection procedure A (enrichment in Bolton broth) and procedure B (enrichment in Preston broth), on at least four occasions. The plating was done on mCCD and Butzler agar.

Table 2. Outline and results of stability testing under transport conditions for proficiency test No. 35, 2023.

Test occasion	Storage conditions [a]	Test methods [b]	Number of samples tested	Detection rate
Before dispatch [c]	Best case	A + B + VC	4 (2 for VC) from each batch of vials with *Campylobacter* = 24	A: 75 % B: 96 %
Before dispatch [c]	Worst case	A + B + VC	4 (2 for VC) from each batch of vials with *Campylobacter* = 24	A: 0 % B: 46 %
Just after dispatch	Best case	A + B	The complete test = 17 [d]	A: 55 % B: 100 %
1 week after dispatch	Worst case	A + B + VC	2 (or 1) from each batch of vials with *Campylobacter* = 22 [d]	A: 45 % B: 100 %

[a] **Best case** transport conditions: 5 °C for 24 h, **worst case** transport conditions: 5 °C for 24 h, 15 °C for 24 h, and 5 °C for 24 h.

[b] Detection procedure according to ISO 10272-1:2017 and ISO 10272-1:2017/Amd 1:2023: **A** enrichment in Bolton broth, **B** enrichment in Preston broth. The detection procedures were applied in samples of chicken skin mixed with vials. **VC**: viable count of vials with *Campylobacter*.

[c] The tests before dispatch were performed with a 0.5 $\log_{10}$ cfu lower concentration of the bacteria (per test portion) than in the final proficiency test.

[d] Only one SVA074 vial (sample No. 11 and 26) was included in these tests.

The detection tests were performed before dispatch in simulated “best case” transport conditions (5 °C for 24 h) and “worst case” transport conditions (5 °C for 24 h, 15 °C for 24 h, and 5 °C for 24 h) altogether with viable counts on blood agar. Dilutions were adjusted to achieve the desired levels indicated above.

The tests of the final PT were performed just after dispatch under "best case" conditions and one week after dispatch (after the last date for start of analysis by the participants) under "worst case" conditions. At the last occasion, the stability of vials with *Campylobacter* stored under "worst case" conditions was also checked by performing viable count on blood agar.

The viable counts showed that the levels were lower after storage of vials under transport conditions, especially in high level samples in "worst case" conditions, but reasonably homogenous in vials from the same batch tested at the same occasion (SD < 0.15 $\log_{10}$ cfu). However, the instability of the vials did not affect the detection rate when performing the tests with the recommended procedure B, enrichment in Preston broth, which was 100 % after storage in both transport conditions. A poor performance was noted for procedure A in all tests both before and after dispatch, probably due to current problems with production of the Bolton broth.

Distribution of the proficiency test

The PT was distributed from the EURL on the 20th of March, 2023. The samples were placed in styrofoam boxes along with freezing blocks. The styrofoam boxes were packed in cardboard boxes for transport and were sent from the EURL using courier service.

Each participant received a package containing 18 numbered vials, each containing freeze-dried material with or without *Campylobacter* spp., and 18 numbered boot sock samples. A Micro-T-Log was included in each package to record the temperature every second hour during transport.

Of the 32 participating NRLs, 27 received the PT within one day after the packages had been dispatched from the EURL, four NRLs within two days, and one NRL after three days (Table 3).

The PT analyses were recommended to be started as soon as possible after the arrival and at the latest on the 24th of March, 2023. All results had to be reported in the Questback Essentials system by the 24th of April, 2023. Instructions for preparation of the samples from the vials and matrix were included in the packages and were also sent out by e-mail a few days before the PT distribution. Until start of analysis, boot sock samples and vials were recommended to be stored at cold temperature (between 1 °C and 8 °C). If the analysis was not started the same day as arrival, the vials were recommended to be stored at –20 °C or –70 °C.

Table 3. Dates of arrival and start of analysis of proficiency test No. 35, 2023.

Arrival	Number of NRLs n=32 [a]	Start of analysis	Number of NRLs n=32 [a]
21st of March	27	21st of March	8
22nd of March	4	22nd of March	12
23rd of March	1	23rd of March	6
		24th of March	5
		28th of March	1

[a] One additional NRL received and started analysis of the test but did not report final results.

Methods for analysis

The NRLs were recommended to follow ISO 10272-1:2017 for performing the PT but were allowed to use another method if their standard laboratory procedure followed a different method. They were recommended to, as far as possible, use the same procedures as for routine samples. If not normally analysing boot sock samples, they were suggested to use procedure B according to ISO 10272-1:2017, enrichment in Preston broth. The amount of material provided allowed the laboratories to use both direct and enrichment procedures if this was of interest to them.

Campylobacter spp. should be incubated in a microaerobic atmosphere, with oxygen content of 5 % ± 2 % and carbon dioxide 10 % ± 3 %. The appropriate microaerobic atmosphere can be obtained by using commercially available microaerobic incubators, commercial gas-generating kits, or by using gas-jars, filled with the appropriate gas mixture prior to incubation. Of the 32 participating NRLs, 21 reported using gas-generating kits, eight microaerobic incubators, and six the Anoxomat® system. Some of the NRLs used more than one system.

Assessment of performance in detection and identification

The NRLs' performance in sensitivity in detection (of *Campylobacter*-positive samples in total and in low-level and high-level samples separately), sensitivity in identification, and accuracy in detection of *Campylobacter*-positive and -negative samples were calculated from the final results as reported by each participant.

The **sensitivity** was calculated based on the NRLs ability to correctly detect *Campylobacter* spp. and identify *Campylobacter* species in the samples containing *Campylobacter*. Correct detection of all *Campylobacter*-positive samples (in the low- and high-level category, respectively), resulted in a sensitivity in detection of 100 %. Correct identification of all *Campylobacter* species in positive samples in which *Campylobacter* spp. were detected resulted in a sensitivity in identification of 100 %.

For determining the performance in detection of negative *Campylobacter* samples, the **specificity** was calculated for each NRL. Correct identification of all samples without *Campylobacter* as non-*Campylobacter* samples resulted in a specificity of 100 %.

The **accuracy** was also calculated, giving an overall performance of the results of correct detection of *Campylobacter* spp. in samples with *Campylobacter* and correct identification of samples without *Campylobacter* as non-*Campylobacter* samples. The accuracy was calculated as total number of correct detection results divided by total number of samples.

A combined five-level grading scale for **performance in detection** was based on the number of correct results of detection for the three categories of samples (low-level *Campylobacter*-positive samples, high-level *Campylobacter*-positive samples, and *Campylobacter*-negative samples) according to Table 4. The cut-off for good **performance in identification** of *Campylobacter* species was set to 85.0 %.

Table 4. The minimum number of correct results (*Campylobacter* detected or not detected) needed for each combined performance grade, and the corresponding measures of sensitivity (Se), specificity (Sp), and accuracy (Acc), in proficiency test No. 35, 2023. Performance scoring below any of the limits for the performance grade *Needs improvement* was graded as *Poor*.

	Category of samples			*Measures on the lower limit of each grade (%)*				
Performance grade	**Low level (n = 6)**	**High level (n = 6)**	**Negative (n = 6)**	**Se low**	**Se high**	**Se total**	**Sp**	**Acc**
Excellent	6	6	6	100.0	100.0	100.0	100.0	100.0
Good	4	5	6	66.7	83.3	75.0	100%	83.3
Acceptable	3	4	5	50.0	66.7	58.3	83.3	66.7
Needs improvement	2	3	4	33.3	50.0	41.7	66.7	50.0

Results

Detection and species identification of *Campylobacter*

The PT was distributed to 33 NRLs and 32 reported the results of the analysis.

According to the instructions, analysis of the samples should be started as soon as possible after arrival and no later than four days after dispatch. Eight NRLs started the analysis the day after the samples were dispatched from the EURL, twelve NRLs two days after, six NRLs three days after, five NRLs four days after, and one NRL eight days after (Table 3).

Of the 32 NRLs reporting results, 31 followed ISO 10272-1:2017 (13 in its original version and 18 including the newly published amendment ISO 10272-1:2017/Amd 1:2023) for detection of *Campylobacter* spp., and one NRL used another culture method. All except two NRLs performed the suggested enrichment procedure in Preston broth. Fifteen NRLs used direct plating, in one case as the only procedure but in all other cases in addition to enrichment procedures. One NRL performed enrichment in Bolton broth (in addition to Preston enrichment) and one in CampyFood® broth. Other additional procedures used for detection were polymerase chain reaction (PCR) of the mixed sample or the enrichment broth (3) and direct plating on blood agar with a 0.8 µm filter (1). Sixteen NRLs used one

procedure only, nine NRLs two procedures, four NRLs three procedures, and one NRL four procedures for the detection part.

Thirty NRLs used mCCD agar whereof 21 plated on at least one additional medium. Other media used for plating were Butzler agar (9), CampyFood® agar (4), Karmali agar (3), Preston agar (2), Skirrow agar (2), RAPID'*Campylobacter* agar BioRad (2), CASA® agar (1), CHROMagar™ *Campylobacter* (1), CAT agar (1), and Chromo *Campylobacter* agar (1).

The presumptive *Campylobacter* colonies were confirmed by typical microscopic morphology and motility, positive oxidase test, lack of aerobic growth at 25 °C and/or molecular methods, mostly matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) or PCR. Twenty of the 32 NRLs used microscopic examination as part of the confirmation procedure. Nineteen NRLs used oxidase test, and 16 NRLs used aerobic growth at 25 °C. In 13 cases the latter two tests were combined. Twenty NRLs used MALDI-TOF MS for confirmation, in eleven cases combined with additional techniques other than microscopic examination. Six NRLs used one or more PCR assays, in five cases combined with additional techniques other than microscopy. Three NRLs reported to have used the multiplex PCR assay published by Wang et al. (2002).

The isolated *Campylobacter* spp. were identified by biochemical tests and/or molecular methods, mostly MALDI-TOF MS or polymerase chain reaction (PCR). The biochemical tests included detection of catalase, hippurate hydrolysis, indoxyl acetate hydrolysis, sensitivity to cephalotin, and hydrogen sulphide production in triple sugar iron medium.

One NRL did not perform species identification because no suspected *Campylobacter* colonies were identified. Of the 31 NRLs that performed species identification of confirmed or suspected *Campylobacter* colonies, 21 used MALDI-TOF MS, in seven cases combined with other techniques. Ten NRLs used one or more PCR assays for the species identification, in eight cases combined with other techniques. Seven NRLs reported to have used or adapted the multiplex PCR assay published by Wang et al. (2002). Other protocols reported by more than one NRL were the PCR assays by Denis et al. (1999) and Best et al. (2003). Twelve NRLs used biochemicals tests (at least detection of catalase), in eight cases combined with MALDI-TOF MS and/or PCR.

Twenty NRLs used one technique only (a set of biochemical tests and/or tests of growth regarded as one technique), ten NRLs combined two techniques, and one NRL three techniques.

Of the 32 NRLs, twenty-one reported correct results of detection, i.e. correct identification of the twelve samples with *Campylobacter* and the six samples without *Campylobacter* (Figure 1). Two NRLs did not detect *Campylobacter* in any of the samples. Twenty-five NRLs reported correct species in all samples where *Campylobacter* spp. had been detected, whereas five NRLs reported at least one misidentification of and/or not being able to identify correctly detected *Campylobacter*.

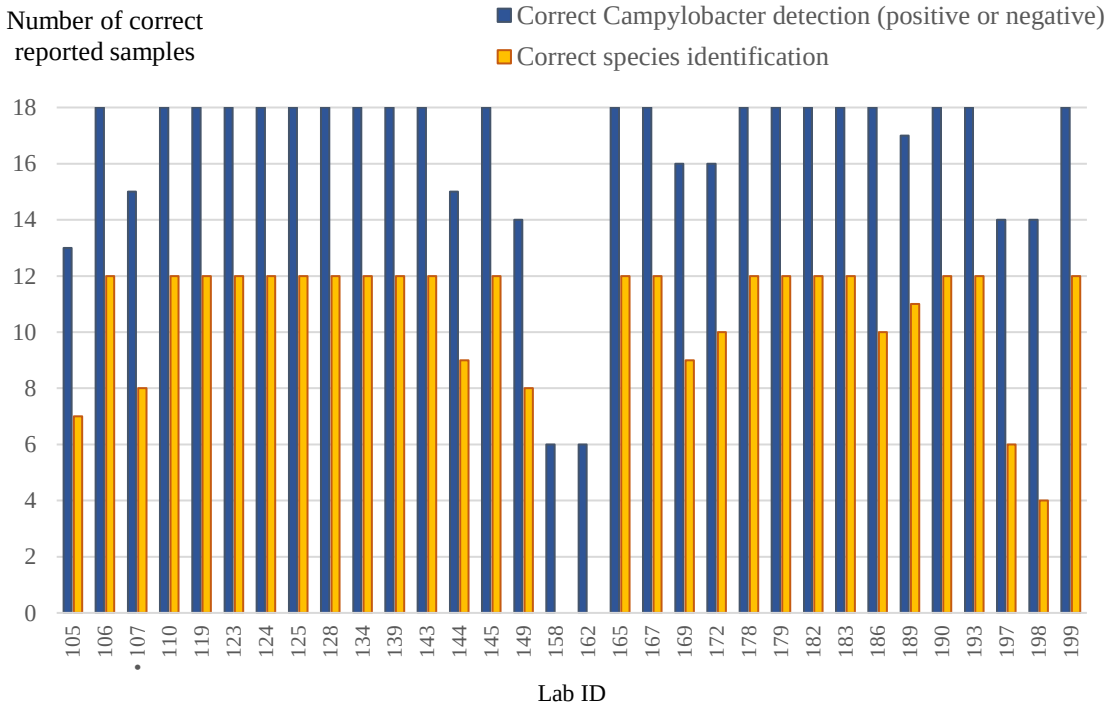

Figure 1. Distribution of correct results by 32 NRLs participating in proficiency test No. 35, 2023, in the detection and species identification of *Campylobacter* spp. in pig faeces. A dot • denotes a false positive result.

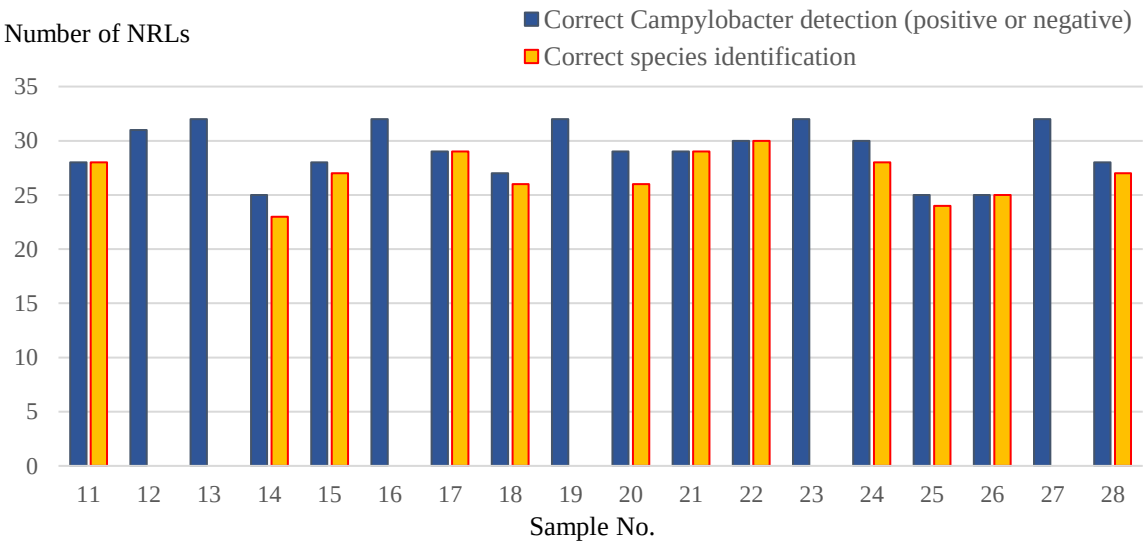

Figure 2. Number of the 32 NRLs participating in proficiency test No. 35, 2023, that correctly reported results in the detection and species identification of *Campylobacter* in 18 boot sock samples.

All 32 NRLs reported correct results of detection for five of the six *Campylobacter*-negative samples, whereas one false positive was reported for sample No. 12 (Figure 2, Table 5). The number of false negative results reported for samples containing *Campylobacter* varied from two to seven, or zero to five with the results from the two NRLs that failed to detect any *Campylobacter* excluded. The highest number of false negative results were reported for low-level samples containing *C. coli* (sample No. 14 and 25) and *C. jejuni* (sample No. 26).

There were three misidentifications of sample No. 20, two of sample No. 24, and one each of sample No. 14 and 18. Evaluation of the reported *Campylobacter* species from individual NRLs revealed that mix-up of samples was a possible explanation in all seven cases of misidentification.

Table 5. Results of detection and species identification in 18 boot sock samples from chicken in proficiency test No. 35, 2023.

Sample No.	Bacterial species in vial	*Campylobacter jejuni*	*Campylobacter coli*	*Campylobacter lari*	*Campylobacter* spp. but unable to identify species	Growth of other, not *Campylobacter*	No growth at all
11	*Campylobacter jejuni*	**28**				3	1
12	*Escherichia coli*	1				**30**	**1**
13	–					**16**	**16**
14	*Campylobacter coli*	1	**23**		1	7	
15	*Campylobacter lari*			**27**	1	4	
16	*Escherichia coli*					**31**	**1**
17	*Campylobacter lari*			**29**		3	
18	*Campylobacter lari*		1	**26**		5	
19	*Escherichia coli*					**29**	**3**
20	*Campylobacter lari*	3		**26**		2	1
21	*Campylobacter coli*		**29**			3	
22	*Campylobacter jejuni*	**30**				2	
23	–					**17**	**15**
24	*Campylobacter jejuni*	**28**		2		2	
25	*Campylobacter coli*		**24**		1	4	3
26	*Campylobacter jejuni*	**25**				6	1
27	–					**21**	**11**
28	*Campylobacter coli*		**27**		1	3	1

Performance in detection and species identification of *Campylobacter* spp.

Of the 32 participating NRLs, 25 NRLs (23 Member State NRLs, MS-NRLs) fulfilled the criterion for excellent or good performance in detection of *Campylobacter*, and three (two MS-NRLs) scored below the acceptable limit (Table 6). Twenty-six NRLs fulfilled the criterion for excellent or good performance in identification of *Campylobacter* spp., and three (one MS-NRL) either scored below the acceptable limit or did not present a result because of poor performance in the detection part (Table 7).

Table 6. Combined performance grades in detection of *Campylobacter* spp. in proficiency test No. 35, 2023.

Combined performance in detection of *Campylobacter* spp.		
Grade	**Number of NRLs (%) All NRLs, n=32**	**Number of NRLs (%) MS-NRLs, n=27**
Excellent	21 (66)	19 (70)
Good	4 (13)	4 (15)
Acceptable	4 (13)	2 (7)
Needs improvement	1 (3)	1 (4)
Poor	2 (6)	1 (4)

Table 7. Overall performance of NRLs' sensitivity in correct species identification of *Campylobacter* in proficiency test No. 35, 2023.

Performance in identification of *Campylobacter* spp.			
Grade	**Sensitivity**	**Number of NRLs (%) All NRLs, n=32**	**Number of NRLs (%) MS-NRLs, n=27**
Excellent	95.1–100 %	25 (78)	24 (89)
Good	85.0–95.0 %	1 (3)	1 (4)
Acceptable	70.0–84.9 %	3 (9)	1 (4)
Needs improvement	57.0–69.9 %	0 (0)	0 (0)
Poor	<57.0 %	1 (3)	0 (0)
No result [a]		2 (6)	1 (4)

[a] Two NRLs did not detect any *Campylobacter* spp. in the samples.

All performance parameters for detection and identification of *Campylobacter* spp. in boot sock samples for all participants are presented in Table 8.

Participants using both enrichment in Preston broth and direct plating had a somewhat higher detection rate (92 %) in low level *Campylobacter* samples than participants using enrichment in Preston broth only (79 %, or 84 % with one NRL that failed to detect any *Campylobacter* at all excluded), whereas the detection rate in high level *Campylobacter* samples did not differ between these groups. Still, 10 of the 16 NRLs using enrichment in Preston broth only detected 100 % of the *Campylobacter*-positive samples.

Table 8. The sensitivity (Se), specificity (Sp), and accuracy (Acc) in detecting *Campylobacter* and non-*Campylobacter* spp., the combined performance grades in detection, and the sensitivity in identification (Se id) of *Campylobacter* spp. for 32 NRLs participating in proficiency test No. 35, 2023. The performance grades in detection were based on minimum limits for sensitivity in detection of low-level samples (Se low), sensitivity in detection of high-level samples (Se high) and specificity (Sp). Green shadowed cells indicate acceptable grades: *Excellent*, *Good* and *Acceptable*, and red shadowed cells indicate grades below the acceptable limit: *Needs improvement* and *Poor*.

Lab id	Se low	Se high	Se total	Sp	Acc	Performance grade in detection	Se id
105	50 %	67 %	58 %	100 %	72 %	Acceptable	100 %
106	100 %	100 %	100 %	100 %	100 %	Excellent	100 %
107	67 %	100 %	83 %	83 %	83 %	Acceptable	80 %
110	100 %	100 %	100 %	100 %	100 %	Excellent	100 %
119	100 %	100 %	100 %	100 %	100 %	Excellent	100 %
123	100 %	100 %	100 %	100 %	100 %	Excellent	100 %
124	100 %	100 %	100 %	100 %	100 %	Excellent	100 %
125	100 %	100 %	100 %	100 %	100 %	Excellent	100 %
128	100 %	100 %	100 %	100 %	100 %	Excellent	100 %
134	100 %	100 %	100 %	100 %	100 %	Excellent	100 %
139	100 %	100 %	100 %	100 %	100 %	Excellent	100 %
143	100 %	100 %	100 %	100 %	100 %	Excellent	100 %
144	67 %	83 %	75 %	100 %	83 %	Good	100 %
145	100 %	100 %	100 %	100 %	100 %	Excellent	100 %
149	33 %	100 %	67 %	100 %	78 %	Needs improvement	100 %
158	0 %	0 %	0 %	100 %	33 %	Poor	–
162	0 %	0 %	0 %	100 %	33 %	Poor	–
165	100 %	100 %	100 %	100 %	100 %	Excellent	100%
167	100 %	100 %	100 %	100 %	100 %	Excellent	100%
169	83 %	83 %	83 %	100 %	89 %	Good	90%
172	67 %	100 %	83 %	100 %	89 %	Good	100%
178	100 %	100 %	100 %	100 %	100 %	Excellent	100%
179	100 %	100 %	100 %	100 %	100 %	Excellent	100%
182	100 %	100 %	100 %	100 %	100 %	Excellent	100%
183	100 %	100 %	100 %	100 %	100 %	Excellent	100%
186	100 %	100 %	100 %	100 %	100 %	Excellent	83%
189	83 %	100 %	92 %	100 %	94 %	Good	100%
190	100 %	100 %	100 %	100 %	100 %	Excellent	100%
193	100 %	100 %	100 %	100 %	100 %	Excellent	100%
197	50 %	83 %	67 %	100 %	78 %	Acceptable	75%
198	50 %	83 %	67 %	100 %	78 %	Acceptable	50%
199	100 %	100 %	100 %	100 %	100 %	Excellent	100%

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