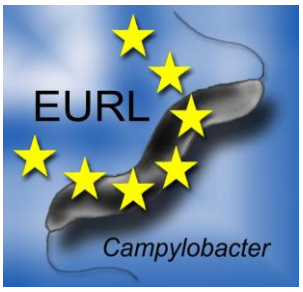

EURL-*CAMPYLOBACTER*

REPORT

PROFICIENCY TEST NUMBER 24

Detection and species identification of *Campylobacter* spp.

Contents

Abbreviations

C.	*Campylobacter*
cfu	colony forming units
ed.	edition
EU	European Union
EURL	European Union reference laboratory
FN	false negative
FP	false positive
ISO	International Organization for Standardization
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-deoxylate (agar)
MS	member state
NRL	national reference laboratory
PCR	polymerase chain reaction
PT	proficiency test
spp.	species

Introduction

Proficiency test (PT) number 24 on detection and species identification of *Campylobacter* was organised by the EU reference laboratory (EURL) for *Campylobacter* in March 2019. The PT included detection and species identification of *Campylobacter* spp. in up to 24 samples mixed with the freeze-dried contents of vials with or without *Campylobacter* (Table 1). The objective was to assess the performance of the NRLs to detect and identify *Campylobacter* species in minced chicken meat and/or boot sock samples.

Table 1. Bacteria in the vials and living bacteria added to samples of minced chicken meat (sample No. 11–20) and boot sock samples (sample No. 21–34) in proficiency test No. 24 (2019).

	Sample No.	Bacterial species in vial	Batch No.	Level [a] (log_{10} cfu/vial)	Bacterial species added to sample
Minced chicken meat	11	*Campylobacter lari*	SVA016	4.38 (high)	*Candida albicans*
	12	*Campylobacter jejuni* [b]	SVA021	4.28 (high)	
	13	*Campylobacter coli*	SVA023	2.93 (low)	
	14	Negative	SLV289	–	
	15	Negative	SLV289	–	
	16	*Campylobacter coli*	SVA022	3.45 (high)	
	17	*Campylobacter lari*	SVA017	3.27 (low)	
	18	*Campylobacter jejuni* [b]	SVA027	2.02 (low)	
	19	*Campylobacter jejuni* [b]	SVA025	3.20 (low)	
	20	*Campylobacter jejuni* [b]	SVA025	3.20 (low)	
Sock samples	21	*Campylobacter jejuni* [b]	SVA025	3.20 (low)	*Escherichia coli*
	22	Negative	SLV289	–	*Escherichia coli*
	23	*Campylobacter jejuni* [b]	SVA027	2.02 (low)	
	24	*Campylobacter jejuni* [b]	SVA021	4.28 (high)	*Escherichia coli*
	25	*Campylobacter lari*	SVA017	3.27 (low)	
	26	*Campylobacter jejuni* [b]	SVA025	3.20 (low)	
	27	*Campylobacter lari*	SVA016	4.38 (high)	*Escherichia coli*
	28	*Campylobacter coli*	SVA022	3.45 (high)	
	29	Negative	SLV289	–	
	30	*Campylobacter coli*	SVA023	2.93 (low)	*Escherichia coli*
Educational	31	*Campylobacter lanienae*	SVA019	3.75	
	32	*Campylobacter helveticus*	SVA026	6.10	
	33	*Campylobacter upsaliensis*	SVA018	4.47	
	34	*Campylobacter jejuni* [b] + *Campylobacter lari*	SVA015	4.48 (in total)	

[a] Total quantity of *Campylobacter* in each vial.
[b] 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) *C. jejuni*, *C. coli*, *C. lari* and *C. 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 methods and/or by molecular methods.
- Species identification of *Campylobacter*: Identification of thermotolerant *Campylobacter* species with biochemical methods and/or by molecular methods.

Outline of the test

The PT contained three sets of samples: ten core samples (No. 11 to 20) of minced chicken meat, ten core samples (No. 21 to 30) of boot socks and four educational samples (No. 31 to 34) of boot socks. Each participating NRL chose at least one set of core samples to receive, with the other core sample set and the educational samples as voluntary parts. The core sock samples were composed to mimic sock samples taken in a chicken house at a farm with conventional rearing (birds kept indoors), and the educational samples in a chicken pen at a farm with ecological or free-range rearing (birds kept outdoors).

Thirty-three national reference laboratories (NRLs) in 25 EU member states (some member states have more than one NRL) and in Iceland, Norway, and Switzerland received the proficiency test. The core samples of minced chicken meat were received by 23 NRLs, the core samples of boot socks by 22 NRLs and the educational samples by 28 NRLs. Thirty-one of the 33 NRLs reported that they were accredited for detection of *Campylobacter* and 22 were also accredited for enumeration of *Campylobacter*.

Preparation of the matrices

The chicken meat, caecal material and 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 very low general level of *Campylobacter*-positive flocks (less than 5 % during the previous year) and no positive flocks at all for two months before taking out and sending broiler carcasses to the EURL. All used materials tested negative for presence of *Campylobacter*. The chicken meat and caeca were freeze-stored until preparation of the PT.

Preparation of minced chicken meat

The freeze-stored chicken meat was thawed at 4 °C, grinded and refreezed. The day before distribution of the PT, the minced chicken meat was thawed at 4 °C, and an overnight culture with *Candida albicans* was prepared. On the day of dispatch, the minced chicken meat was mixed with the overnight culture, homogenised and divided in 120 g portions, one for each participant.

Preparation of boot sock samples

Four days before the distribution of the PT, an overnight culture with *Escherichia coli* was prepared. Three days before dispatch, freeze-stored caeca were thawed, cut, placed in a stomacher bag and mixed with Cary Blair transport medium. For samples with added background flora (Table 1), the overnight culture was mixed with the caecum suspension. For samples without added background flora, serum broth of the same volume was added to the caecum suspension. For each sample, 20 ml of the suspension (with or without background flora) were added to a plastic bag with a boot sock. A small amount of litter material was also added to each sample. The sock samples were stored at 4 °C over the weekend.

Production and quality control of bacterial cultures

The vials with freeze-dried bacterial cultures used in the PT were produced and tested for homogeneity and stability by the EURL (all *Campylobacter*-containing samples) or the Swedish National Food Agency (negative samples). The non-*Campylobacter* strains (*Escherichia coli* and *Candida albicans*) used as background flora in the matrices were tested for use as live cultures.

Each combination of vial and matrix was prepared and tested according to ISO 10272-1:2017 at least three times: before dispatching, just after dispatching and four days after dispatching, i.e. at the last time for start of the analysis by the participants. The samples with minced chicken meat were tested according to procedure A (enrichment in Bolton broth), and the boot sock samples were tested according to procedure A, procedure B (enrichment in Preston broth), and procedure C (direct plating).

Distribution of the proficiency test

The PT samples were distributed from the EURL on 11th of March, 2019. The samples were placed in foam boxes along with freezing blocks. The foam boxes were packed in cardboard boxes for transportation and were sent from the EURL using courier service.

Each participant received a package containing one or more of three sets of samples:

Core samples, minced chicken meat:

- ten numbered vials; each containing freeze-dried material with or without *Campylobacter* spp., and
- one plastic bag with minced chicken meat (ca 120 g), to be divided into 10 g portions, one for each of the ten vials.

Core samples, boot sock samples:

- ten numbered vials; each containing freeze-dried material with or without *Campylobacter* spp., and
- ten boot sock samples in plastic bags, one for each of the ten vials.

Educational samples, boot sock samples:

- four numbered vials; each containing freeze-dried material with or without *Campylobacter* spp., and
- four boot sock samples in plastic bags, one for each of the four vials.

Twenty-four NRLs received the PT within one day after the packages had been dispatched from the EURL, and nine NRLs two days after. A Micro-T-Log was included in each shipment to record the temperature every second hour during transport.

The PT analyses were recommended to be started as soon as possible after the arrival and at the latest on 15th of March, 2019. All results had to be reported in the Questback Essentials system by 15th of April, 2019. Instructions describing possible procedures for preparation of the samples from the vials and matrices were included in the packages, and were also sent out by e-mail a few days before he PT distribution.

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 also recommended to use the same sample preparation procedure as they would use for routine samples of the same kind at their laboratory. The instructions included examples of sample preparation procedures for both enrichment and direct plating procedures.

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 33 NRLs, 19 reported using gas-generating kits, nine microaerobic incubators, seven the Anoxomat® system and two other methods (zip-lock bags filled with gas and jars filled with gas mixture). Some of the NRLs used more than one system.

Assessment of performance in detection and identification

The NRLs' performance of detection and species identification were assessed based on the results of analysis of the core samples of minced chicken meat and boot socks, respectively. For the educational samples, only the overall performance of all NRLs was assessed, not the performance of each individual NRL.

For defining good performance in detection of *Campylobacter* spp. and identification of *Campylobacter* species, calculation of each of the NRLs' ability to correctly detect *Campylobacter* spp. and identify *Campylobacter* species, i.e. the **sensitivity**, was performed. Correct detection of all *Campylobacter* positive samples 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%. The cut-off for good performance of detection/identification of *Campylobacter* species was set to 85.0%.

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. The cut-off for good performance was set to 90%.

Since there were only two *Campylobacter*-negative samples in each set of results for which the performance assessment was done, the specificity was not assessed.

Results

Detection and species identification of *Campylobacter* in minced chicken meat

The minced chicken meat samples No. 11 to 20 in proficiency test number 24 were distributed to 23 NRLs and 22 of them reported the results of the analysis. Four NRLs started the analysis the day after the samples were dispatched from the EURL, ten NRLs started the analysis two days after, two NRLs three days after, four NRLs four days after, one NRL six days after and one NRL two weeks after the samples were distributed from the EURL. According to the instructions, analysis of the samples should be started as soon as possible after arrival and no later than four days after the dispatching.

Nineteen NRLs reported to have followed ISO 10272-1:2017 for detection of *Campylobacter* spp. in minced chicken meat, and three NRLs used other methods. All 22 NRLs used a procedure including enrichment, and two of them used direct plating as well. Bolton broth was used for the enrichment by 16 NRLs, Preston broth by 7 NRLs (two NRLs used both Bolton and Preston broth), and one NRL used Campy Food Bouillon for the enrichment. Twenty-one NRLs did the plating on mCCD agar, and 16 plated on at least one additional medium. Other media used for plating were CampyFood agar (5), Preston agar (4), CASA agar (3), Skirrow agar (3), Abeyta-Hunt-Bark agar (1), Butzler agar (1) and CHROMagar (1).

The isolated *Campylobacter* spp. were identified by biochemical methods and/or molecular methods, mostly matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) or polymerase chain reaction (PCR). The biochemical methods included detection of catalase, hippurate hydrolysis, indoxyl acetate hydrolysis, and sensitivity to nalidixic acid and cephalotin.

Twelve of the 22 NRLs reported that they used MALDI-TOF MS for the species identification, in seven cases in combination with other techniques. Eight NRLs used PCR assays, in three cases in combination with other techniques. Four NRLs reported to have used the multiplex PCR assay published by Wang *et al.* (2002). Nine NRLs used biochemicals methods (at least detection of catalase), in six cases in combination with MALDI-TOF MS or PCR. One NRL used whole genome sequencing (WGS) for the species identification. One NRL reported to have used the API® Campy system in addition to other biochemical tests. Other complementary methods, each used by one NRL, were VIDAS® and 16S rDNA sequencing.

Twelve NRLs used one technique only (a set of biochemical tests regarded as one technique, and the API® Campy as one technique), nine NRLs combined two techniques, and one NRL used three techniques for the species identification.

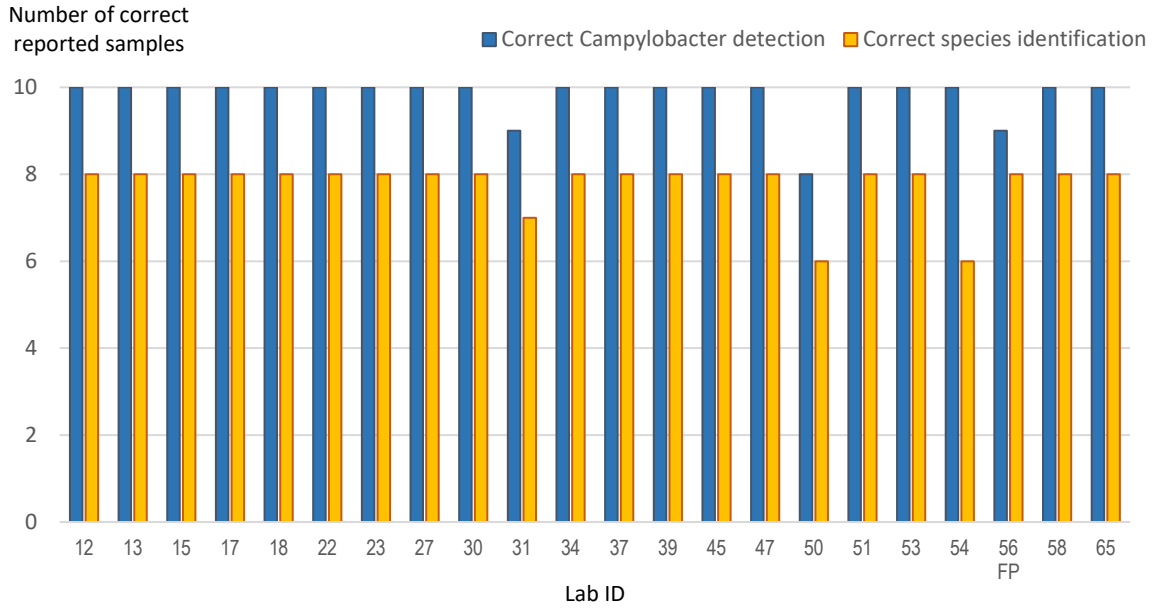

Figure 1. Distribution of correctly reported results by 22 NRLs participating in proficiency test No. 24 (2019) in the detection and species identification of *Campylobacter* spp. in minced chicken meat samples No. 11–20. One false positive result is marked with FP.

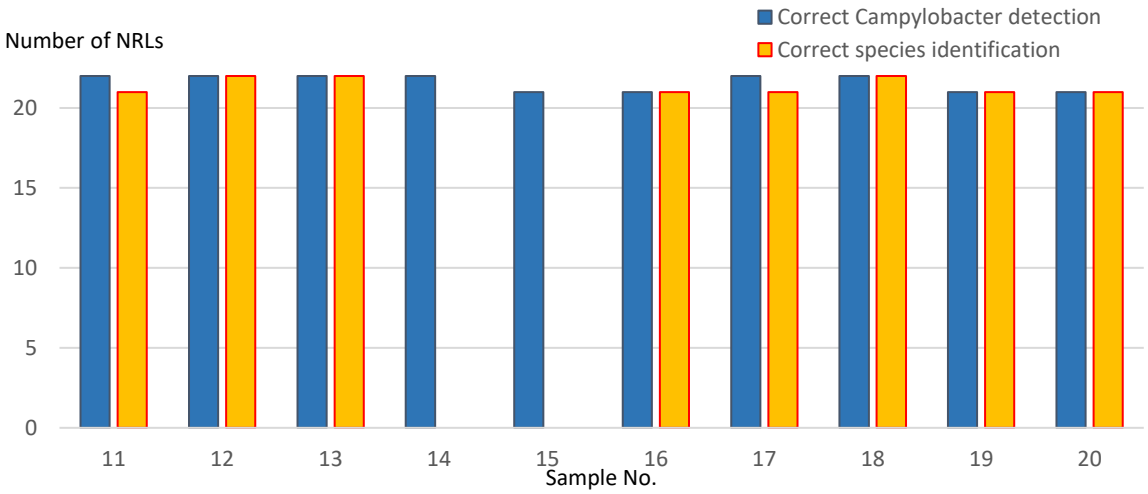

Figure 2. Distribution of number of NRLs participating in proficiency test No. 24 (2019) that correctly reported results in the detection and species identification of *Campylobacter* in minced chicken meat.

Of the 22 NRLs, 19 reported correct results of detection, i.e. correct identification of the eight samples **with** *Campylobacter* and the two samples **without** *Campylobacter* (Figure 1). One false positive result was reported, of sample No. 15. Regarding the species identification, 19 of the 22 NRLs reported correct species in all the eight samples that had been inoculated with *Campylobacter* spp. Eighteen NRLs reported correct results of both detection and species identification.

For six of the ten samples, five samples which contained *Campylobacter* and one sample that did not, all 22 NRLs reported correct detection results (Figure 2, Table 2). Three of the five *Campylobacter*-positive samples that were correctly detected by all NRLs were also correctly identified by all 22 NRLs, two as *Campylobacter jejuni* and one as *Campylobacter coli*. No sample was incorrectly detected or identified by more than one NRL.

Table 2. Results of detection and species identification of samples No. 11–20 in proficiency test No. 24 (2019).

Sample No.	**Bacterial species**	Level (log_{10} cfu/sample)	*C. jejuni*	*C. coli*	*C. lari*	*Campylobacter* spp. but unable to identify species	Growth of other, not *Campylobacter*	No growth at all
11	*Campylobacter lari*	4.38			21	1		
12	*Campylobacter jejuni*	4.28	22					
13	*Campylobacter coli*	2.93		22				
14	Negative						7	15
15	Negative			1			8	13
16	*Campylobacter coli*	3.45		21				1
17	*Campylobacter lari*	3.27			21	1		
18	*Campylobacter jejuni*	2.02	22					
19	*Campylobacter jejuni*	3.20	21				1	
20	*Campylobacter jejuni*	3.20	21				1	

Detection and species identification of *Campylobacter* in boot sock samples

The boot sock samples No. 21 to 30 in proficiency test number 24 were distributed to 22 NRLs and all of them reported the results of the analysis. One NRL started the analysis the day after the samples were dispatched from the EURL, thirteen NRLs started the analysis two days after, three NRL three days after, and five NRLs four days after the samples were distributed from the EURL.

Twenty-one NRLs reported to have followed ISO 10272-1:2017 for detection of *Campylobacter* spp. in boot sock samples, and one NRL used another method. Twenty NRLs used a procedure including enrichment, and five of them used direct plating as well. Ten NRLs used Bolton broth and ten NRLs Preston broth for the enrichment. One NRL did only direct plating, and one NRL did not report if enrichment was used or not. All 22 NRLs did the plating on mCCD agar, and 16 plated on at least one additional medium. Other media used for plating were CampyFood agar (4), Preston agar (4), Karmali agar (3), Abeyta-Hunt-Bark agar (1), Butzler agar (1), CASA agar (1), CAT agar (1), CHROMagar (1) and Skirrow agar (1).

The isolated *Campylobacter* spp. were identified by biochemical methods and/or molecular methods, PCR, MALDI-TOF MS or 16S rDNA sequencing. The biochemical methods included detection of catalase, hippurate hydrolysis, indoxyl acetate hydrolysis, and sensitivity to nalidixic acid and cephalotin.

Fourteen of the 22 NRLs reported that they used PCR assays for the species identification, in eleven cases in combination with other techniques. Nine NRLs reported to have used the multiplex PCR assay published by Wang *et al.* (2002). Another protocol reported to be used or adapted by more than one NRL was the PCR assay by Denis *et al.* (1999). Nine NRLs used MALDI-TOF MS, in five cases in combination with other techniques. Eleven NRLs used biochemicals methods (at least detection of catalase), in eight cases in combination with MALDI-TOF MS and/or PCR. One NRL reported to have used the API® Campy system in addition to other biochemical tests, and one NRL used 16S rDNA sequencing for the identification.

Nine NRLs used one technique only (a set of biochemical tests regarded as one technique, and the API® Campy as one technique), twelve NRLs combined two techniques, and one NRL used three techniques for the species identification.

Of the 22 NRLs, 11 reported correct results of detection, i.e. correct identification of the eight samples **with** *Campylobacter* and the two samples **without** *Campylobacter* (Figure 3). One false positive result was reported, of sample No. 29. Regarding the species identification, the same 11 NRLs reported correct species in all the eight samples that had been inoculated with *Campylobacter* spp. Hence, 11 NRLs reported correct results of both detection and species identification.

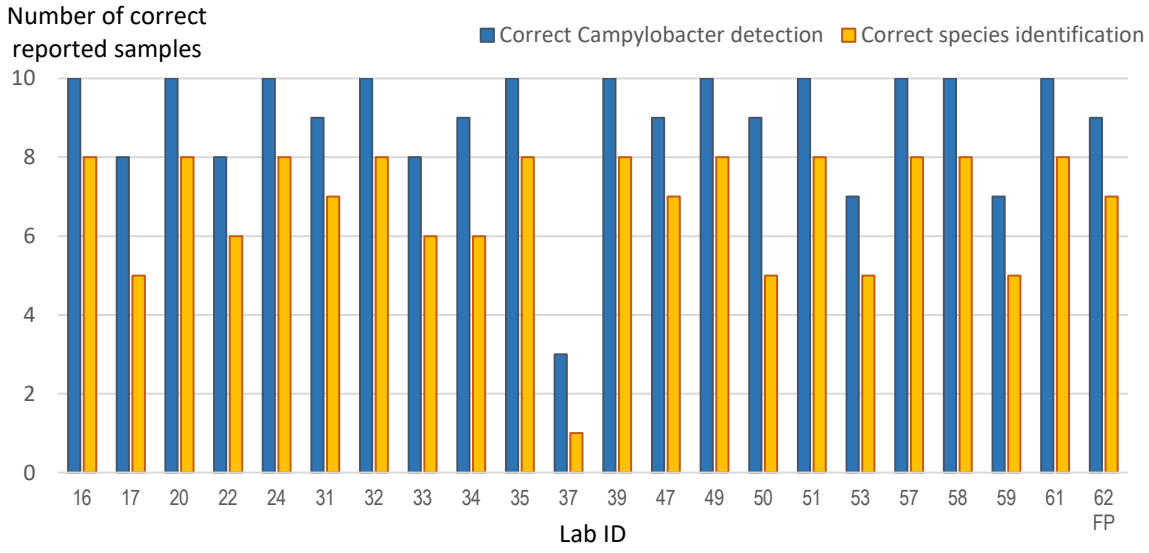

Figure 3. Distribution of correctly reported results by 22 NRLs participating in proficiency test No. 24 (2019) in the detection and species identification of *Campylobacter* spp. in boot sock samples No. 21–30. One false positive result is marked with FP.

The eight *Campylobacter*-positive samples were correctly detected by 17 to 21 NRLs, i.e. one to five false negative results (FN) were reported for each sample (Figure 4, Table 3). The samples that caused most difficulties were samples number 21 (5 FN), 23 (5 FN) and 26 (4 FN), all containing *Campylobacter jejuni* at a low level. However, most of these false negative results were reported by the same three NRLs, which underperformed at the PT as a whole.

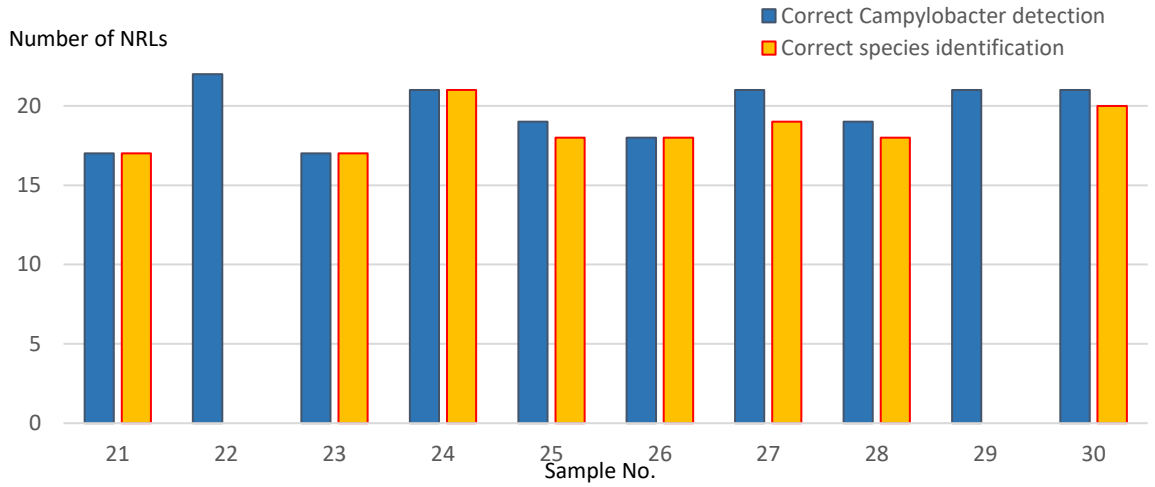

Figure 4. Distribution of number of NRLs participating in proficiency test No. 24 (2019) that correctly reported results in the detection and species identification of *Campylobacter* in boot sock samples.

Table 3. Results of detection and species identification of samples No. 21–30 in proficiency test No. 24 (2019).

Sample No.	**Bacterial species**	Level (log_{10} cfu/sample)	*C. jejuni*	*C. coli*	*C. lari*	*Campylobacter* spp. but unable to identify species	Growth of other, not *Campylobacter*	No growth at all
21	*Campylobacter jejuni*	3.20	17				3	2
22	Negative						18	4
23	*Campylobacter jejuni*	2.02	17				3	2
24	*Campylobacter jejuni*	4.28	21					1
25	*Campylobacter lari*	3.27		1	18		2	1
26	*Campylobacter jejuni*	3.20	18				2	2
27	*Campylobacter lari*	4.38	1	1	19		1	
28	*Campylobacter coli*	3.45		18	1		2	1
29	Negative		1				15	6
30	*Campylobacter coli*	2.93		20	1			1

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

The performance (sensitivity and accuracy) in detection and identification of *Campylobacter* spp. in minced chicken meat and boot sock samples, respectively, are presented in Table 4.

Table 4. The performance (sensitivity and accuracy) in detecting *Campylobacter* and non-*Campylobacter* spp. and the performance (sensitivity) in identification of *Campylobacter* spp. of the 33 NRLs participating in proficiency test No. 24 (2019). Shadowed cells indicate performance below 100%. Green shadowed cells indicate grades Good (bright green) and Acceptable (pale green). Red shadowed cells indicate grades below the acceptable limit.

	Minced chicken meat (sample No 11–20)			**Boot sock samples (samples No. 21–30)**		
Lab id	**Sensitivity in detection**	**Accuracy in detection**	**Sensitivity in species identification**	**Sensitivity in detection**	**Accuracy in detection**	**Sensitivity in species identification**
12	100%	100%	100%	–	–	–
13	100%	100%	100%	–	–	–
15	100%	100%	100%	–	–	–
16	**–**	**–**	**–**	100%	100%	100%
17	100%	100%	100%	75%	80%	83%
18	100%	100%	100%	–	–	–
20	–	–	–	100%	100%	100%
22	100%	100%	100%	75%	80%	100%
23	100%	100%	100%	–	–	–
24	–	–	–	100%	100%	100%
27	100%	100%	100%	–	–	–
30	100%	100%	100%	–	–	–
31	88%	90%	100%	88%	90%	100%
32	–	–	–	100%	100%	100%
33	–	–	–	75%	80%	100%
34	100%	100%	100%	88%	90%	86%
35	–	–	–	100%	100%	100%
37	100%	100%	100%	13%	30%	100%
39	100%	100%	100%	100%	100%	100%
45	100%	100%	100%	–	–	–
47	100%	100%	100%	88%	90%	100%
49	–	–	–	100%	100%	100%
50	75%	80%	100%	88%	90%	71%
51	100%	100%	100%	100%	100%	100%
53	100%	100%	100%	63%	70%	100%
54	100%	100%	75%	–	–	–
56	100%	90%	100%	–	–	–
57	–	–	–	100%	100%	100%
58	100%	100%	100%	100%	100%	100%
59	–	–	–	63%	70%	100%
61	–	–	–	100%	100%	100%
62	–	–	–	100%	90%	88%
65	100%	100%	100%	–	–	–

The overall results of the NRLs' sensitivity in detection and identification of *Campylobacter* spp. in minced chicken meat and boot sock samples, respectively, were categorized on a five-level grading scale. Also, the accuracy, the combined result of the detection of *Campylobacter* spp. and identification of non-*Campylobacter* samples, was categorized on a five-level grading scale for each matrix.

For **minced chicken meat**, 21 NRLs (19 MS-NRLs) fulfilled the criterion for excellent or good performance in detection of *Campylobacter* and none scored below the acceptable limit (Table 5). Twenty-one NRLs (19 MS-NRLs) fulfilled the criterion for excellent performance in identification of *Campylobacter* spp., and none scored below the acceptable limit (Table 6). Regarding accuracy, 21 laboratories (19 MS-NRLs) fulfilled the criterion for excellent or good performance, and none scored below the acceptable limit (Table 7).

Table 5. Overall performance of NRLs' sensitivity in correct detection of *Campylobacter* spp. in minced chicken meat, proficiency test No. 24 (2019).

Detection of *Campylobacter* spp. in minced chicken meat (sample No. 11–20)			
Grade	**Sensitivity**	**Number of NRLs (%) All NRLs, n=22**	**Number of NRLs (%) MS-NRLs, n=20**
Excellent	95.1–100%	20 (91%)	18 (90%)
Good	85.0–95.0%	1 (5%)	1 (5%)
Acceptable	70.0–84.9%	1 (5%)	1 (5%)
Needs improvement	57.0–69.9%	0 (0%)	0 (0%)
Poor	<57.0%	0 (0%)	0 (0%)

Table 6. Overall performance of NRLs' sensitivity in correct species identification of *Campylobacter* in minced chicken meat, proficiency test No. 24 (2019).

Identification of *Campylobacter* spp. in minced chicken meat (sample No. 11–20)			
Grade	**Sensitivity**	**Number of NRLs (%) All NRLs, n=22**	**Number of NRLs (%) MS-NRLs, n=20**
Excellent	95.1–100%	21 (95%)	19 (93%)
Good	85.0–95.0%	0 (0%)	0 (0%)
Acceptable	70.0–84.9%	1 (5%)	1 (5%)
Needs improvement	57.0–69.9%	0 (0%)	0 (0%)
Poor	<57.0%	0 (0%)	0 (0%)

Table 7. Overall performance of NRLs' accuracy in correctly detecting *Campylobacter* positive and negative samples in minced chicken meat, proficiency test No. 24 (2019).

Detection of *Campylobacter* positive and negative samples in minced chicken meat (sample No. 11–20)			
Grade	**Accuracy**	**Number of NRLs (%) All NRLs, n=22**	**Number of NRLs (%) MS-NRLs, n=20**
Excellent	95.1–100%	19 (86%)	17 (85%)
Good	90.0–95.0%	2 (9%)	2 (10%)
Acceptable	80.0–89.9%	1 (5%)	1 (5%)
Needs improvement	70.0–79.9%	0 (0%)	0 (0%)
Poor	<70.0%	0 (0%)	0 (0%)

The overall median sensitivity in correctly detecting *Campylobacter* in minced chicken meat was 100% (50% Central Range (CR): 100%–100%) and in correctly identifying *Campylobacter* spp. 100% (50% CR: 100%–100%). The overall median accuracy in detection of *Campylobacter* spp. and identification of non-*Campylobacter* samples was 100% (50% CR: 100%–100%).

For **boot sock samples**, 16 NRLs (14 MS-NRLs) fulfilled the criterion for excellent or good performance in detection of *Campylobacter* and three MS-NRLs scored below the acceptable limit (Table 8). Twenty NRLs (18 MS-NRLs) fulfilled the criterion for excellent or good performance in identification of *Campylobacter* spp., and none scored below the acceptable limit (Table 9). Regarding accuracy, 16 laboratories (14 MS-NRLs) fulfilled the criterion for excellent or good performance, and three MS-NRLs scored below the acceptable limit (Table 10).

Table 8. Overall performance of NRLs' sensitivity in correct detection of *Campylobacter* spp. in boot sock samples, proficiency test No. 24 (2019).

Detection of *Campylobacter* spp. in boot sock samples (sample No. 21–30)			
Grade	**Sensitivity**	**Number of NRLs (%) All NRLs, n=22**	**Number of NRLs (%) MS-NRLs, n=20**
Excellent	95.1–100%	12 (55%)	11 (55%)
Good	85.0–95.0%	4 (18%)	3 (15%)
Acceptable	70.0–84.9%	3 (14%)	3 (15%)
Needs improvement	57.0–69.9%	2 (9%)	2 (10%)
Poor	<57.0%	1 (5%)	1 (5%)

Table 9. Overall performance of NRLs' sensitivity in correct species identification of *Campylobacter* in boot sock samples, proficiency test No. 24 (2019).

Identification of *Campylobacter* spp. in boot sock samples (sample No. 21–30)			
Grade	**Sensitivity**	**Number of NRLs (%) All NRLs, n=22**	**Number of NRLs (%) MS-NRLs, n=20**
Excellent	95.1–100%	18 (82%)	16 (80%)
Good	85.0–95.0%	2 (9%)	2 (10%)
Acceptable	70.0–84.9%	2 (9%)	2 (10%)
Needs improvement	57.0–69.9%	0 (0%)	0 (0%)
Poor	<57.0%	0 (0%)	0 (0%)

Table 10. Overall performance of NRLs' accuracy in correctly detecting *Campylobacter* positive and negative samples in boot sock samples, proficiency test No. 24 (2019).

Detection of *Campylobacter* positive and negative samples in boot sock samples (sample No. 21–30)			
Grade	**Accuracy**	**Number of NRLs (%) All NRLs, n=22**	**Number of NRLs (%) MS-NRLs, n=20**
Excellent	95.1–100%	11 (50%)	10 (50%)
Good	90.0–95.0%	5 (23%)	4 (20%)
Acceptable	80.0–89.9%	3 (14%)	3 (15%)
Needs improvement	70.0–79.9%	2 (9%)	2 (10%)
Poor	<70.0%	1 (5%)	1 (5%)

The overall median sensitivity in correctly detecting *Campylobacter* in boot sock samples was 100% (50% CR: 78.1%–100%) and in correctly identifying *Campylobacter* spp. 100% (50% CR: 100%–100%). The overall median accuracy in detection of *Campylobacter* spp. and identification of non-*Campylobacter* samples was 95.0% (50% CR: 82.5%–100%).

Detection and species identification of *Campylobacter* in educational samples

The educational samples No. 31 to 34 in proficiency test number 24 were distributed to 28 NRLs and 27 of them reported the results of the analysis. Twenty-three NRLs reported to have followed ISO 10272-1:2017 for detection of *Campylobacter* spp. in the educational samples, and four NRLs used other methods. Twenty-four NRLs used a procedure including enrichment, and seven of them used direct plating as well. Bolton broth was used for the enrichment by 15 NRLs, Preston broth by 10 NRLs (two NRLs used both Bolton and Preston broth), and one NRL used Campy Food Bouillon for the enrichment. Two NRLs did only direct plating, and one NRL did not report if enrichment was used or not. Twenty-six NRLs did the plating on mCCD agar, and 21 plated on at least one additional medium. Other media used for plating were CampyFood agar (6), Preston agar (6), Skirrow agar (4), Karmali agar (3), Butzler agar (2), CASA agar (1), CAT agar (1), CHROMagar (1) and blood agar with 0.45 µm filter (1).

The isolated *Campylobacter* spp. were identified by biochemical methods and/or molecular methods, PCR, MALDI-TOF MS or 16S rDNA sequencing. The biochemical methods included detection of catalase, hippurate hydrolysis, indoxyl acetate hydrolysis, sensitivity to nalidixic acid and cephalotin, H_2S production in triple sugar iron medium, growth in NaCl 3.5%, growth in glycine 1%, growth on MacConkey agar, growth on nutrient agar, and nitrate reduction.

Sixteen of the 27 NRLs reported that they used PCR assays for the species identification, in twelve cases in combination with other techniques. Nine NRLs reported to have used the multiplex PCR assay published by Wang *et al.* (2002). Another protocol reported to be used or adapted by more than one NRL was the PCR assay by Denis *et al.* (1999). Fourteen NRLs used MALDI-TOF MS, in eight cases in combination with other techniques. Twelve NRLs used biochemicals methods (at least detection of catalase), in ten cases in combination with MALDI-TOF MS and/or PCR. One NRL reported to have used the API® Campy system in addition to other biochemical tests. Three NRLs used 16S rDNA sequencing for one or more samples.

Twelve NRLs used one technique only (a set of biochemical tests regarded as one technique, and the API® Campy as one technique), eleven NRLs combined two techniques, and four NRLs used three techniques for the species identification.

The results of detection and species identification of *Campylobacter* in the educational boot sock samples are presented in Table 11.

Table 11. Results of detection and species identification of educational boot sock samples No. 31 to 34 in proficiency test No. 24 (2019). Shadowed cells indicate correct species identification: green for correct answers regarding all present species and yellow for correct answer regarding one of two present species in sample No. 34.

Sample No.	*Campylobacter* species	*C. jejuni*	Both *C. jejuni* and *C. lari*	*C. lari*	*C. upsaliensis*	*C. helveticus*	*C. lanienae*	*C. cuniculorum*	*Campylobacter* spp. but unable to identify species	No *Campylobacter*
31	*Campylobacter lanienae*	1		2			11		7	6
32	*Campylobacter helveticus*					9		1		17
33	*Campylobacter upsaliensis*				17				1	9
34	*Campylobacter jejuni* + *Campylobacter lari*	11	11	4					1	

The overall sensitivity in detection of *Campylobacter* spp. in samples No. 31–34 was 70.4% (Table 12). The overall sensitivity in correct species identification was 73.0%.

To calculate the performance rate of both detection and species identification for all NRLs together, a scoring system was used. Each correct detection result was given score 1 and each correct identification result for samples No. 31, 32 and 33 score 1. Each correct identification result for sample No. 34 (i.e. *C. jejuni* or *C. lari*) was given score 0.5. The sum of the scores was divided by two times the total number of samples (i.e. the maximum score possible). The overall performance rate for the educational samples was 60.9% (Table 12).

Table 12. Overall sensitivity in detection and species identification and overall performance rate for 27 NRLs in analysis of educational samples in proficiency test No. 24 (2019).

Sample No.	*Campylobacter* species	Sensitivity in detection	Sensitivity in species identification	Combined performance rate of detection and identification
31	*C. lanienae*	77.8%	52.4%	59.3%
32	*C. helveticus*	37.0%	90.0%	35.2%
33	*C. upsaliensis*	66.7%	94.4%	64.8%
34	*C. jejuni* + *C. lari*	100.0% [a]	68.5% [b]	84.3%
All		70.4%	73.0%	60.9%

[a] The sensitivity in specific detection of *C. jejuni* and *C. lari* was 81.5% and 55.6%, respectively.

[b] The sensitivity in species identification for sample No. 34 was calculated as the total number of correct identifications (11+4+11×2) divided by the total number of possible correct identifications for the samples in which *Campylobacter* was detected (27×2). The sensitivity in species identification reflect both detection and species identification for this sample.

The median performance rate, calculated for each NRL, was 62.5%. Four NRLs had a performance rate of 100% on the educational samples, i.e., they reported correct results on both detection and species identification for all four samples. Two NRLs had all correct results except on sample No. 34 where they reported only one of the two species included, which gave a performance rate of 93.8%. No grades were assigned for analysis of the educational samples, which were included in the PT for educational purposes.

Summary of proficiency test number 24, 2019

Proficiency test No. 24 on detection and species identification of *Campylobacter* was distributed on March 11, 2019. The PT included detection and species identification of *Campylobacter* spp. in up to 24 samples. The objective was to assess the performance of the NRLs to detect and identify *Campylobacter* species in minced chicken meat and/or boot sock samples. Thirty-three NRLs in 25 EU member states and in Iceland, Norway, and Switzerland participated in the proficiency test.

The PT contained three sets of samples: ten core samples of minced chicken meat, ten core samples of boot socks and four educational samples of boot socks. Each participating NRL chose at least one set of core samples to receive, with the other core sample set and the educational samples as voluntary parts. The core sock samples were composed to mimic sock samples taken in a chicken house at a farm with birds kept indoors, and the educational samples in a chicken pen at a farm with birds kept outdoors.

Most NRLs used the recommended method ISO 10272-1:2017 for analysing the samples, with a large variation in which procedure or combination of procedures that had been followed, for both matrices. The majority of the NRLs fulfilled the criteria for excellent or good performance for detection and identification of *Campylobacter* spp. in minced chicken meat and/or boot sock samples, although a somewhat lower detection rate was reported in boot sock samples, where 16/22 NRLs showed an excellent or good performance.

The results of the educational samples were not included in the performance evaluation. According to the results, the most challenging sample for detection was sample no. 32 containing *Campylobacter helveticus*, and for identification sample no. 31 containing *Campylobacter lanienae*.

References

ISO 10272-1:2017: Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of *Campylobacter* spp. – Part 1: Detection method. International Organization for Standardization.

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