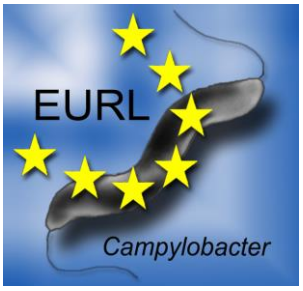

EURL-*CAMPYLOBACTER*

REPORT

PROFICIENCY TEST NUMBER 22

Detection and species identification of *Campylobacter* spp. in chicken faecal swab samples

Contents

Abbreviations

C.	*Campylobacter*
cfu	colony forming units
ed.	edition
EU	European Union
EURL	European Union reference laboratory
ISO	International Organization for Standardization
$\log_{10}$	logarithm to base 10 (common logarithm)
mCCD	modified charcoal-cefoperazone-deoxylate (agar)
MS	member state
NMKL	Nordic Committee on Food Analysis (Nordisk metodikkomite for levnedsmidler)
NRL	national reference laboratory
PCR	polymerase chain reaction
PT	proficiency test
spp.	species

Introduction

Proficiency test (PT) number 22 on detection and species identification of *Campylobacter* in chicken faecal swab samples was organised by the EU reference laboratory (EURL) for *Campylobacter* in March 2018. The PT included detection and species identification of *Campylobacter* spp. in 22 chicken faecal swab samples with or without *Campylobacter* mixed with the freeze-dried content 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 chicken faecal swab samples.

Table 1. Bacteria in the vials and added to swab samples in proficiency test No. 22 (2018).

	Sample No.	Bacterial species in vial	Batch No.	Level*	Bacterial species added to sample
Core	11	*Campylobacter coli*	SVA005	High	
	12	*Campylobacter coli*	SLV221	Low	
	13	*Campylobacter jejuni***	SVA009	Low	*Escherichia coli*
	14	Negative	SLV151		
	15	Negative	SLV151		
	16	Negative	SLV151		
	17	*Campylobacter jejuni***	SVA014	High	*Escherichia coli*
	18	Negative	SLV151		*Escherichia coli*
	19	*Campylobacter jejuni***	SVA009	Low	
	20	*Campylobacter jejuni***	SVA014	High	*Candida****
	21	*Campylobacter lari*	SVA012	High	*Escherichia coli*
	22	Negative	SLV151		*Candida****
	23	*Campylobacter jejuni***	SVA004	High	
	24	*Campylobacter coli*	SVA005	High	*Escherichia coli*
	25	*Campylobacter lari*	SVA011	Low	*Candida****
	26	Negative	SLV151		*Escherichia coli*
	27	*Campylobacter jejuni***	SVA013	Low	*Escherichia coli*
	28	*Campylobacter lari*	SVA011	Low	
Educational	29	*Campylobacter upsaliensis*	SVA008	High	
	30	*Campylobacter lari*	SLV299	High	
	31	*Campylobacter coli*	SVA006	Low	*Campylobacter jejuni***
	32	*Campylobacter hyointestinalis*	SVA003	High	

*Vials of high level contained a total of 4–6 $\log_{10}$ cfu and vials of low level 2–3 $\log_{10}$ cfu.
**All *Campylobacter jejuni* strains were hippurate positive.
****Candida glabrata* according to MALDI-TOF MS, isolated from faeces from cattle.

Eighteen samples (No. 11 to 28) were core samples, and the NRLs' performance of both detection and species identification were assessed based on the results of analysis of these samples. Four samples (No. 29 to 32) were educational samples. Analysis of the educational samples was voluntary, and the results were not included in the assessment of performance. The core samples were composed to mimic chicken faecal or caecal swabs taken at a farm with conventional rearing (birds kept indoors), and the educational samples at a farm with ecological or free-range rearing (birds kept outdoors).

Thirty-one national reference laboratories (NRLs) in 25 EU member states (some member states have more than one NRL) and in Former Yugoslav Republic of Macedonia, Iceland, Norway, and Switzerland received the proficiency test. Twenty-nine of the 31 NRLs reported that they were accredited for detection of *Campylobacter* and 20 were also accredited for enumeration of *Campylobacter*.

Terms and definitions

- *Campylobacter* spp.: Thermophilic *Campylobacter* spp., foremost *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 *Campylobacter* species with biochemical methods and/or by molecular methods.

Outline of the proficiency test

Preparation of e-swabs for the proficiency test

The chicken caeca used in the PT were obtained from a broiler producer that had not delivered any *Campylobacter* positive flocks to slaughter for more than one year. The birds were slaughtered at a slaughterhouse with a very low general level of *Campylobacter* positive flocks (3.7 % during 2017) and no positive flocks at all for two months before taking out and sending broiler carcasses to the EURL. Chicken skin and caecal samples from the broiler flock tested negative for presence of *Campylobacter*. The caeca were freeze-stored until preparation of the PT.

Swab samples were prepared three days before the distribution of the PT to resemble chicken cloacal swab samples. Overnight cultures of bacteria (Table 1) were prepared. Caeca were cut and placed in a stomacher bag and mixed with Cary Blair transport medium. A dilution of each overnight culture was mixed with the caecum suspension to enhance the background flora. Each e-swab was emptied of its existing content and filled with 1 ml of caecum suspension (with or without added bacteria according to Table 1).

Production of vials and quality control of the samples

The vials with freeze-dried bacterial cultures used in the PT were produced and tested for stability and homogeneity by the Swedish National Food Agency or the EURL. Before sending the PT to the NRLs, each combination of vial and e-swab was prepared and tested in duplicates according to ISO 10272-1:2017, procedure C (direct plating) and procedure B (enrichment in Preston broth of two different batches), respectively. Subsequently, all samples were tested four times each for growth of *Campylobacter* spp. and background flora.

Distribution of the proficiency test

The PT samples were distributed from the EURL on 5th of March, 2018. 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:

- twenty-two numbered vials, each containing freeze dried material (with or without *Campylobacter* spp.), and
- twenty-two numbered e-swabs, each containing chicken caecal material (with or without *Campylobacter* spp. and/or other species) in Cary Blair broth.

Twenty-three NRLs received the PT within one day after the packages had been dispatched from the EURL, and eight 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 the results had to be reported in the Questback Essentials system by 23rd of April, 2018. The NRLs were recommended to follow ISO 10272-1:2017 for performing PT 22. However, if their standard laboratory procedure followed a different method, they were allowed to use that method for the test. Instructions for preparation of the the samples from the vials and e-swabs were included in the packages.

Cultivation and incubation

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 31 NRLs, twenty reported using of gas-generating kits, ten microaerobic incubators, five 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.

Assessing the performance of the NRLs

Good performance in detection and identification of *Campylobacter* spp.

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 of 100%. Correct identification of all *Campylobacter* species in positive samples in which *Campylobacter* spp. were detected resulted in a sensitivity of 100%. The cut-off for good performance of detection/identification of *Campylobacter* species was set to 85.0%.

For defining good performance in detection of negative *Campylobacter* samples, the **specificity** was calculated for each NRL. Correct identification of samples without *Campylobacter* as non-*Campylobacter* samples resulted in a specificity of 100%. The cut-off for good performance in detection of non-*Campylobacter* samples was set to 83.0%.

The **accuracy rate** 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 rate 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%.

Results

Proficiency test number 22 was distributed to 31 NRLs and all of them reported the results of the analysis. Five NRLs started the analysis the day after the samples were dispatched from the EURL, nineteen NRLs started the analysis two days after, five NRLs three days after and two NRLs four days after the samples were distributed from the EURL.

Detection and species identification of *Campylobacter* in core samples (mandatory)

Twenty-seven NRLs reported to have followed ISO 10272-1:2017 for detection of *Campylobacter* spp. in the 18 core samples. One NRL reported to have used the previous version ISO/TS 10272-1:2006, one NMKL 119, 3rd ed. 2007, and two NRLs other methods. Eighteen NRLs used a procedure including enrichment, and ten of them used direct plating as well. Thirteen laboratories did only direct plating. Of the NRLs that performed enrichment of the samples, eight used Bolton broth only, seven Preston broth only, and two used both Bolton and Preston broth. All 31 NRLs did the plating on mCCD agar, and 20 plated on an additional medium: Preston agar (5), Karmali agar (3), CampyFood agar (3), Skirrow agar (3), Butzler agar (2), CampyCount agar (1), CHROMagar (1), CAT agar (1) or CASA agar (1).

The isolated *Campylobacter* spp. were identified by biochemical methods and/or molecular methods, PCR or matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). The biochemical methods included detection of catalase, hippurate hydrolysis, indoxyl acetate hydrolysis, and sensitivity to nalidixic acid and cephalotin. One NRL reported to have used the API Campy system in addition to other biochemical tests.

Sixteen of the 31 NRLs reported that they used MALDI-TOF MS for the species identification, in six cases combined with one or more other methods. Fourteen NRLs used one or more PCR assays, in eleven cases in combination with other methods. Nine NRLs reported to have used 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). Fifteen NRLs used biochemicals methods (at least detection of catalase), in eleven cases in combination with MALDI-TOF MS and/or PCR.

Sixteen NRLs used one method only (a set of biochemical tests regarded as one method, and the API Campy as one method), fourteen NRLs combined two methods, and one NRL used all three of biochemical tests, MALDI-TOF MS and PCR for the species identification.

Of the 31 NRLs, 22 reported correct results of detection, i.e. correct identification of the 12 samples **with** *Campylobacter* and the 6 samples **without** *Campylobacter* (Figure 1). Regarding the species identification, 21 of the 31 NRLs reported correct species in all the 12 samples that had been inoculated with *Campylobacter* spp. Twenty-one NRLs reported correct results of both detection and species identification.

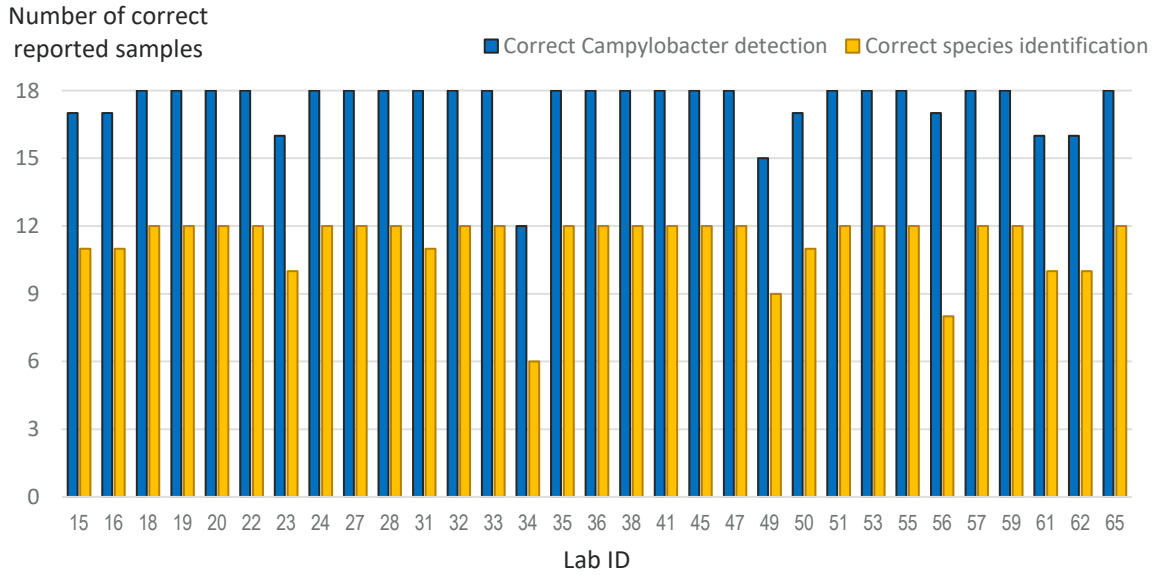

Figure 1. Distribution of correct reported results by 31 NRLs participating in proficiency test No. 22 (2018) in the detection and species identification of *Campylobacter* spp. in chicken faecal swab samples (core samples).

For ten of the 18 samples, four samples which contained *Campylobacter* and the six samples that did not, all 31 NRLs reported correct detection results (Figure 2, Table 2). The four *Campylobacter*-positive samples that were correctly detected by all NRLs were also correctly identified by all 31 NRLs, three as *Campylobacter jejuni* and one as *Campylobacter coli*. The samples that caused most difficulties were samples No. 25 and 28 inoculated with *Campylobacter lari* at a low level.

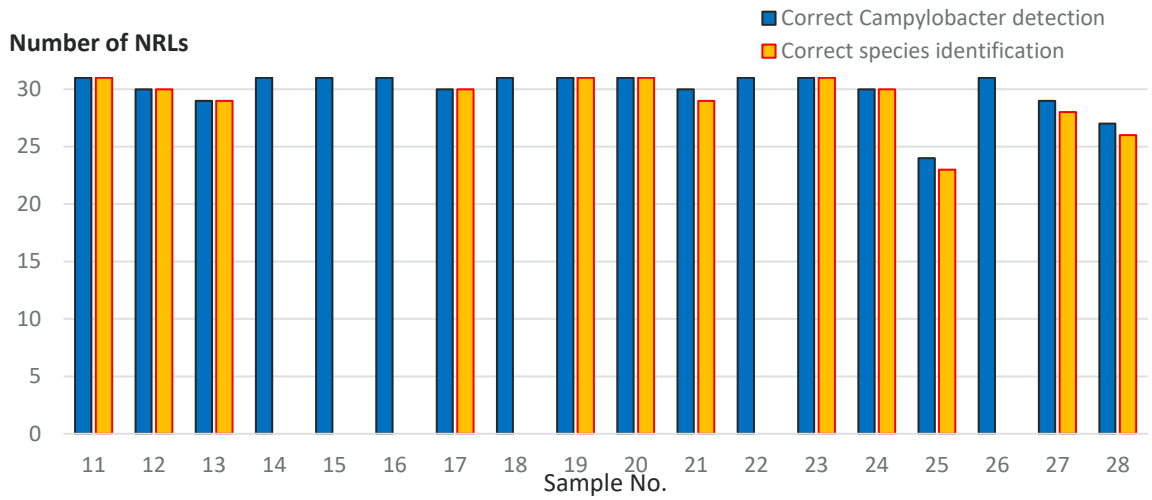

Figure 2. Distribution of number of NRLs participating in proficiency test No. 22 (2018) that correctly reported results in the detection and species identification of *Campylobacter* in chicken faecal swab samples No. 11–28.

Table 2. Results of detection and species identification of samples No. 11–28 in proficiency test No. 22 (2018).

Sample No.	**Bacterial species**	Hippurate hydrolysis	*C. jejuni*	*C. coli*	*C. lari*	*Campylobacter* spp. but unable to identify species	Growth of other, not *Campylobacter*	No growth at all
11	*Campylobacter coli*			31				
12	*Campylobacter coli*			30			1	
13	*Campylobacter jejuni*	+	29				2	
14	Negative						11	20
15	Negative						10	21
16	Negative						13	18
17	*Campylobacter jejuni*	+	30				1	
18	*Escherichia coli*						29	2
19	*Campylobacter jejuni*	+	31					
20	*Campylobacter jejuni*	+	31					
21	*Campylobacter lari*				29	1	1	
22	*Candida* spp.						22	9
23	*Campylobacter jejuni*	+	31					
24	*Campylobacter coli*			30			1	
25	*Campylobacter lari*				23	1	6	1
26	*Escherichia coli**						27	4
27	*Campylobacter jejuni*	+	28		1		2	
28	*Campylobacter lari*				26	1	2	2

*This sample was erroneously reported by one laboratory as *Campylobacter coli*. Because this was proved to be a mistake in filling in the questionnaire only, the result has been corrected.

Good performance in detection and identification of *Campylobacter* spp.

The performance (sensitivity, specificity and accuracy rate) in detecting *Campylobacter*, non-*Campylobacter* spp. and identification of *Campylobacter* spp. is presented in Table 3.

Table 3. The performance (sensitivity, specificity and accuracy rate) in detecting *Campylobacter* and non-*Campylobacter* spp. and the performance (sensitivity) in identification of *Campylobacter* spp. of the 31 NRLs participating in proficiency test No. 22 (2018). 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 criteria.

Lab id	Sensitivity in detection	Specificity in detection	Accuracy rate in detection	Sensitivity in species identification
15	92%	100%	94%	100%
16	92%	100%	94%	100%
18	100%	100%	100%	100%
19	100%	100%	100%	100%
20	100%	100%	100%	100%
22	100%	100%	100%	100%
23	83%	100%	89%	100%
24	100%	100%	100%	100%
27	100%	100%	100%	100%
28	100%	100%	100%	100%
31	100%	100%	100%	92%
32	100%	100%	100%	100%
33	100%	100%	100%	100%
34	50%	100%	67%	100%
35	100%	100%	100%	100%
36	100%	100%	100%	100%
38	100%	100%	100%	100%
41	100%	100%	100%	100%
45	100%	100%	100%	100%
47	100%	100%	100%	100%
49	75%	100%	83%	100%
50	92%	100%	94%	100%
51	100%	100%	100%	100%
53*	100%	100%	100%	100%
55	100%	100%	100%	100%
56	92%	100%	94%	73%
57	100%	100%	100%	100%
59	100%	100%	100%	100%
61	83%	100%	89%	100%
62	83%	100%	89%	100%
65	100%	100%	100%	100%

*This NRL erroneously reported one false positive result because of a mistake in filling in the questionnaire. The result has been adjusted. According to the reported answers, specificity for detection was 83% and the accuracy rate 94%.

The overall sensitivity in detection of *Campylobacter* spp. in samples made from vials containing high and low levels of the target analysed by NRLs using procedures based on direct plating or enrichment is presented in Table 4.

Table 4. Overall sensitivity in detection of *Campylobacter* spp. in chicken faecal samples with high and low levels of the target among the 31 NRLs participating in proficiency test No. 22 (2018). Vials of high level contained a total of 4–6 $\log_{10}$ cfu and vials of low level 2–3 $\log_{10}$ cfu.

Procedure(s) used	Number of NRLs	Sample content	Number of samples	Sensitivity
All	31	*Campylobacter* spp. (all)	372	94.9%
All	31	High level *Campylobacter* spp.	186	98.4%
All	31	Low level *Campylobacter* spp.	186	91.4%
Direct plating only	13	Low level *Campylobacter* spp.	78	88.5%
Enrichment only	8	Low level *Campylobacter* spp.	48	89.6%
Both principles	10	Low level *Campylobacter* spp.	60	96.7%

The overall results of the NRLs' sensitivity in detection of *Campylobacter* and in identification of *Campylobacter* spp. were categorized in a five-grade scoring system. Twenty-six NRLs (23 MS-NRLs) fulfilled the criteria for excellent or good performance for detection of *Campylobacter* and one scored below the acceptable criteria (Table 5). Thirty NRLs (26 MS-NRLs) fulfilled the criteria for excellent or good performance for identification of *Campylobacter* spp., and none scored below the acceptable criteria (Table 6). The overall median sensitivity in correctly detecting *Campylobacter* was 100% (50% Central Range (CR): 91.7%–100%) and in correctly identifying *Campylobacter* spp. 100% (50% CR: 100%–100%).

Table 5. Overall performance of NRLs' sensitivity in correct detection of *Campylobacter* in proficiency test No. 22 (2018).

		Detection of *Campylobacter*	
Grade	Sensitivity	Number of NRLs (%) All NRLs, n=31	Number of NRLs (%) MS-NRLs, n=27
Excellent	95.1–100%	22 (71%)	19 (70%)
Good	85.0–95.0%	4 (13%)	4 (15%)
Acceptable	70.0–84.9%	4 (13%)	3 (11%)
Needs improvement	57.0–69.9%	0 (0%)	0 (0%)
Poor	<57.0%	1 (3%)	1 (4%)

Table 6. Overall performance of NRLs' sensitivity in correct species identification of *Campylobacter* in proficiency test No. 22 (2018).

		Identification of *Campylobacter* spp.	
Grade	Sensitivity	Number of NRLs (%) All NRLs, n=31	Number of NRLs (%) MS-NRLs, n=27
Excellent	95.1–100%	29 (94%)	25 (93%)
Good	85.0–95.0%	1 (3%)	1 (4%)
Acceptable	70.0–84.9%	1 (3%)	1 (4%)
Needs improvement	57.0–69.9%	0 (0%)	0 (0%)
Poor	<57.0%	0 (0%)	0 (0%)

Concerning the specificity (to identify samples without *Campylobacter* as non-*Campylobacter* samples), 31 NRLs (27 MS-NRLs) fulfilled the criteria for excellent performance and none scored below acceptable criteria (Table 7). The overall median specificity in correctly identifying non-*Campylobacter* samples was 100% (50% CR: 100%–100%).

Table 7. Overall performance of NRLs' specificity in correctly identifying samples without *Campylobacter* in proficiency test No. 22 (2018).

		Identification of non-*Campylobacter* samples	
Grade	**Specificity**	**Number of NRLs (%) All NRLs, n=31**	**Number of NRLs (%) MS-NRLs, n=27**
Excellent	95.1–100%	31 (10%)	27 (100%)
Good	83.0–95.0%	0 (0%)	0 (0%)
Acceptable	66.7–82.9%	0 (0%)	0 (0%)
Needs improvement	50.0–66.6%	0 (0%)	0 (0%)
Poor	<50.0%	0 (0%)	0 (0%)

The NRLs' performance of the accuracy rate, the combined result of the detection of *Campylobacter* spp. and identification of non-*Campylobacter* samples, was categorized in a five-grade scoring system (Table 8). Twenty-six laboratories (23 MS-NRLs) fulfilled the criteria for excellent or good performance, and one scored below the acceptable criteria. The overall median accuracy rate in detection of *Campylobacter* spp. and identification of non-*Campylobacter* samples was 100% (50% CR: 94.4%–100%).

Table 8. Overall performance of NRLs' accuracy rate in correctly detecting *Campylobacter* positive and negative samples in proficiency test No. 22 (2018).

		Detection of positive and negative *Campylobacter* samples	
Grade	**Accuracy rate**	**Number of NRLs (%) All NRLS, n=31**	**Number of NRLs (%) MS-NRLs, n=27**
Excellent	95.1–100%	22 (71%)	19 (67%)
Good	90.0–95.0%	4 (13%)	4 (15%)
Acceptable	80.0–89.9%	4 (13%)	3 (11%)
Needs improvement	70.0–79.9%	0 (0%)	0 (0%)
Poor	<70.0%	1 (3%)	1 (4%)

Detection and species identification of *Campylobacter* in educational samples (voluntary)

All 31 NRLs participating in PT 22 also reported results of detection and species identification in the four educational samples (No. 29–32). Most NRLs reported to have used exactly or nearly the same method as for the core samples. Seven NRLs reported additions or modifications of the methods used for detection and/or identification of the educational samples. The results are presented in Table 9.

Table 9. Results of detection and species identification of samples No. 29–32 in proficiency test No. 22 (2018). Shadowed cells indicate correct species identification: bright yellow for totally correct answers and pale yellow for partly correct answers.

Sample No.	*Campylobacter* species	*C. jejuni*	*C. coli*	Both *C. jejuni* and *C. coli*	*C. lari*	*C. upsaliensis*	*C. helveticus*	*C. hyointestinalis*	*C. fetus*	*Campylobacter* spp. but unable to identify species	No *Campylobacter* detected
29	*Campylobacter upsaliensis*	1				9	3			2	16
30	*Campylobacter lari*	1			29						1
31	*Campylobacter coli* / *Campylobacter jejuni**	18	4	8							1
32	*Campylobacter hyointestinalis*	1			1			17		3	8

*The result was faulty reported by one NRL as *C. lari*. Because this was proved to be a mistake in filling in the questionnaire only, the result has been adjusted.

The overall sensitivity in detection of *Campylobacter* spp. in samples No. 29–32 was 79.0% (Table 10). The overall sensitivity in correct species identification was 75.5%.

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 a score of 1 and each correct identification result for samples No. 29, 30 and 32 also a score of 1. Each correct identification result for sample No. 31 (i.e. *C. jejuni* or *C. coli*) was given a score of 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 69.4% (Table 10).

Table 10. Overall sensitivity in detection and species identification and overall performance rate for 31 NRLs analysing the four educational samples in proficiency test No. 22 (2018).

Sample No.	*Campylobacter* species	Sensitivity in detection	Sensitivity in species identification	Combined performance rate of detection and identification
29	*C. upsaliensis*	48.4%	60.0%	38.7%
30	*C. lari*	96.8%	96.7%	95.2%
31	*C. coli* + *C. jejuni*	96.8%	63.3%*	79.0%
32	*C. hyointestinalis*	74.2%	73.9%	64.5%
All		79.0%	75.5%	69.4%

*The sensitivity in species identification for sample No. 31 was calculated as the total number of correct identifications (18+4+8×2) divided by the total number of possible correct identifications for the samples in which *Campylobacter* was detected (30×2).

The median performance rate, calculated for each NRL, was 68.8%. Three 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. Five NRLs had all correct results except on sample No. 31 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 22, 2018

The proficiency test included detection and species identification of *Campylobacter* spp. in 18 core samples, mimicking swabs taken from birds kept indoors. The objective was to assess the performance of the national reference laboratories (NRLs) to detect and identify *Campylobacter* species in chicken faecal swab samples. Four educational samples, mimicking swabs taken from birds kept outdoors, were included as an optional part of the test. Thirty-one NRLs in 25 EU member states and in Former Yugoslav Republic of Macedonia, Iceland, Norway, and Switzerland participated in the proficiency test.

Most NRLs used the recommended method ISO 10272-1:2017 for analysing the samples, but there was a large variation in which procedure or combination of procedures that had been followed. Twenty-six NRLs (23 MS-NRLs) fulfilled the criteria for excellent or good performance for detection of *Campylobacter* and one scored below the acceptable criteria. Thirty NRLs (26 MS-NRLs) fulfilled the criteria for excellent or good performance for identification of *Campylobacter* spp., and none scored below the acceptable criteria.

The results of the educational samples were not included in the performance evaluation. According to the results, the most challenging sample, both for detection and identification, was the sample containing *Campylobacter upsaliensis*.

Overall, the NRLs performed well. The EURL-*Campylobacter* has offered assistance to the MS-NRL with poor performance.

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