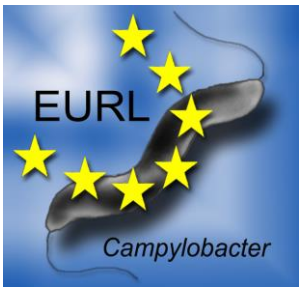

EURL-*CAMPYLOBACTER*

REPORT

PROFICIENCY TEST NUMBER 21

Enumeration (and voluntary detection and species identification) of *Campylobacter* in chicken skin

Contents

Abbreviations

C.	*Campylobacter*
cfu	colony forming units
CR	central range
ed.	edition
EU	European Union
EURL	European Union reference laboratory
ISO	International Organization for Standardization
$\log_{10}$	logarithm to base 10 (common logarithm)
MAD	median absolute deviation
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 21 on detection and enumeration of *Campylobacter* spp. in chicken skin was organised by the EU reference laboratory (EURL) for *Campylobacter* in March 2018. Thirty-seven national reference laboratories (NRLs) in 28 EU member states (some member states have more than one NRL) and in Albania, Former Yugoslav Republic of Macedonia, Iceland, Norway, and Switzerland participated in the PT. The test report and operational details were reported to the EURL from all 37 NRLs. Thirty-six NRLs reported that they were accredited for detection of *Campylobacter* and 27 were also accredited for enumeration of *Campylobacter*. PT 21 included enumeration and voluntary detection of *Campylobacter* in ten chicken skin samples 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 enumerate *Campylobacter* in chicken skin. Detection and species identification of *Campylobacter* were included as a voluntary part of PT 21.

Table 1. Content of the ten vials distributed to the NRLs in proficiency test No. 21 (2018).

Sample No.	Species	Batch No.
1	Negative	151
2	*Campylobacter lari*	248
3	*Campylobacter lari*	299
4	*Escherichia coli*	150
5	*Campylobacter coli* and *Escherichia coli*	221
6	*Campylobacter jejuni**	235
7	*Campylobacter coli*	SVA007
8	*Campylobacter jejuni**	SVA004
9	*Campylobacter jejuni**	SVA010
10	*Campylobacter jejuni**	259

*All *Campylobacter jejuni* strains were hippurate positive.

Terms and definitions

- *Campylobacter* spp.: Thermophilic *Campylobacter* spp., foremost *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis.*
- Enumeration of *Campylobacter*: Determination of the number of *Campylobacter* colony forming units (cfu) per g.
- 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 thermophilic *Campylobacter* species with biochemical methods and/or by molecular methods.

Outline of the proficiency test

Preparation of the chicken skin

The chicken skin used as matrix in the PT was obtained from a broiler producer 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 (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 chicken skin was freeze-stored until distribution of the PT.

Production and quality control of the vials

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, control of *Campylobacter* levels and homogeneity was performed by the EURL for three vials of each batch together with chicken skin. The vials and matrices were spread on duplicate blood agar plates and on single plates of modified charcoal-cefoperazone-deoxylate (mCCD) agar. The results were noted as common logarithm values (log_{10}) for analysis of each tested vial and values for the difference between the highest and lowest values. The samples chosen for the PT included vials with both high and low *Campylobacter* levels, and the maximum difference allowed was 0.50 log_{10} cfu/g.

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:

- ten numbered vials; each containing freeze dried material (with or without *Campylobacter* spp.), and
- one plastic bag with chicken skin (110–120 g), to be divided into 10 g portions, one for each of the ten vials.

Thirty-two NRLs received the PT within one day after the packages had been dispatched from the EURL, and five NRLs two days after (Table 2). A Micro-T-Log was included in each shipment to record the temperature every second hour during transport.

Table 2. Dates of arrival and start of the analysis.

Arrival	Number of NRLs	Start of analysis	Number of NRLs
6th of March	32	6th of March	2
7th of March	5	7th of March	13
		8th of March	2
		9th of March	2
		12th of March	8
		13th of March	3
		14th of March	4
		19th of March	1
		21th of March	1
		26th of March	1

All results had to be reported in the Questback Essentials system by 23rd of April, 2018. The proficiency test was recommended to be started the same week as the PTs were dispatched from the EURL. The NRLs were recommended to follow ISO 10272-2:2017 for performing PT 21. However, if their standard laboratory procedure followed a different method, they were allowed to use that method for the test. Instructions for preparation of an initial dilution of each sample were included in the packages. If the analysis could not be started the same week, the chicken skin was recommended to be stored in −20 °C for up to two weeks and the vials in −20 °C for one week or in −70 °C for two weeks. The dates for the start of analysis are presented in Table 2.

Used methods

Thirty-one NRLs reported to have followed the recommended method of ISO 10272-2:2017. Two NRLs reported to have used the previous version ISO/TS 10272-2:2006, two NMKL 119, 3rd ed. 2007, and two NRLs other methods.

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 37 NRLs, 22 reported using commercial gas-generating kits, eleven microaerobic incubators, six the Anoxomat® system and three other methods (jars filled with gas mixture, zip-lock bags filled with gas or GENbox microaer-generator). Some of the NRLs used more than one system.

Assessing the performance of the NRLs

Good performance in enumeration

The performance in enumeration was assessed by using the median absolute deviation (MAD) method, which is a method that is used to identify outlying counts when fewer than 50 participants undertake an enumeration (ISO/TS 22117:2010). However, also z-scores were calculated and are given separately as some NRLs need to present z-scores for their accreditation. A scoring system was used for assessing MAD from the median value, where results within median value ±2σMAD were given score 2, results between ±2σMAD and ±2.58σMAD were given score 1 and results outside ±2.58σMAD were given score 0. For the *Campylobacter*-negative samples a score of 2 were given when no campylobacters were reported, and a score of 0 when a false positive result was reported.

An overall assessment of all ten enumerations, i.e. *including* the two *Campylobacter*-negative samples, was performed by summarising all the scores for each NRL. A five-grade scoring system was used for the overall assessment: excellent, good, acceptable, needs improvement and poor. "Excellent performance" was considered if all enumerations were within median values ±2σMAD and no campylobacters were reported in the two *Campylobacter*-negative samples, i.e. the total score was 20. "Good performance" was considered if the NRL had a score of 17–19. "Acceptable performance" was considered if the NRL had a score of 14–16. "Needs improvement" were given to NRLs with a score of 12–13 and those with a score of <12 were considered to have a "poor performance".

In addition, an overall assessment (equivalent to assessment in previous years) of the eight enumerations of the *Campylobacter*-positive samples, i.e. *excluding* the two *Campylobacter*-negative samples, was performed by summarising the MAD scores. "Excellent performance" was considered if the total score was 16. "Good performance" was considered if the NRL had a score of 14–15. "Acceptable performance" was considered if the NRL had a score of 12–13. "Needs improvement" were given to NRLs with a score of 10–11 and those with a score of <10 were considered to have a "poor performance".

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

The performance in correctly detecting *Campylobacter* and identifying the species, the sensitivity, was categorized in a five-grade scoring system. The cut-off for good performance was set to 85.0%. For PT 21, only the sensitivity was calculated as there were too few blank samples to calculate the specificity.

Results

Proficiency test number 21 was distributed to 37 NRLs and all of them reported the results of the analysis. Nineteen laboratories started the analyses the same week the samples were dispatched from the EURL, fifteen NRLs the week after, two NRLs two weeks after and one NRL three weeks after the PT was dispatched from the EURL (Table 2).

Enumeration of *Campylobacter* spp. (mandatory)

Of the 37 laboratories, 33 correctly reported *Campylobacter* spp. in all samples with *Campylobacter* spp. and not *Campylobacter* in the samples without *Campylobacter* (Figure 1). Two false positive results were reported by two laboratories, of sample No. 4. Four false negative results were reported, two each of samples No. 2 and 3, by two NRLs.

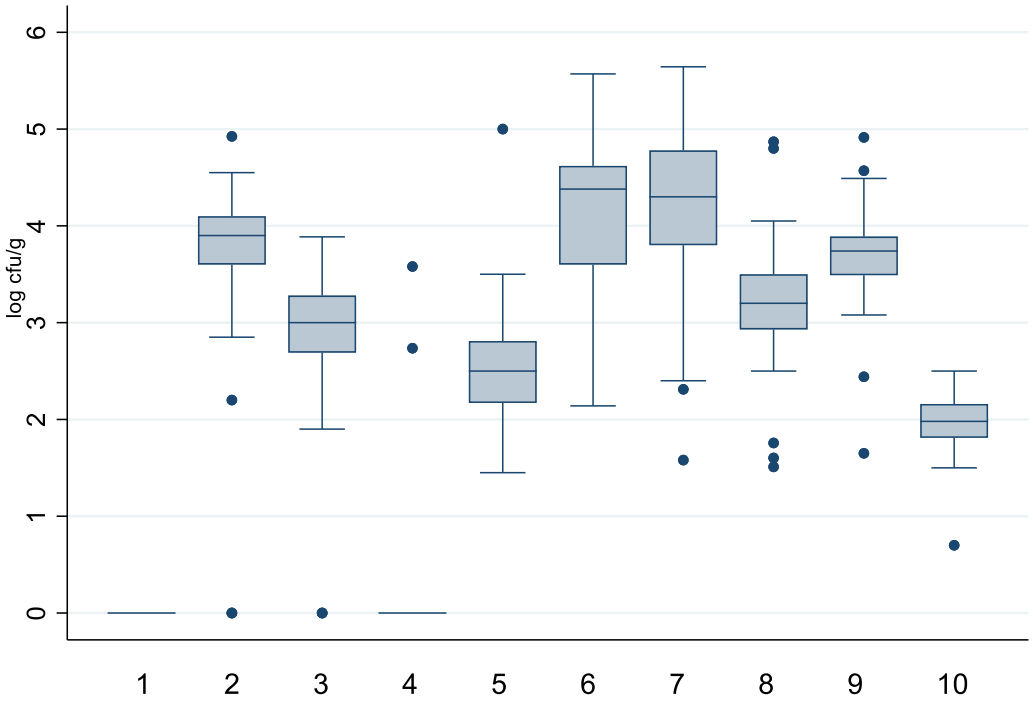

Figure 1. The number (log_{10} cfu/g) of *Campylobacter* spp. reported by 37 laboratories in PT 21 (2018). The samples reported as *Campylobacter* spp. not detected are shown as 0 in the figure.

Good performance in enumeration

The results of using the five-grade scoring system for the overall assessment of the NRLs' enumeration of *Campylobacter* spp. with and without the negative samples are presented in Table 3 and Figure 2.

According to the assessment including all samples, 31 NRLs (28 of the MS-NRLs) fulfilled the criteria for excellent or good performance and three NRLs (one MS-NRL) scored below the acceptable criteria (Table 3 and Figure 2). The overall median percentage of MAD scores was 100% (50% central range (CR): 90.0%–100%).

According to the additional assessment including only the *Campylobacter*-positive samples, 30 NRLs (28 of the MS-NRLs) fulfilled the criteria for excellent or good performance and five NRLs (three MS-NRLs) scored below the acceptable criteria (Table 3). The overall median percentage of MAD scores was 100% (50% CR: 87.5%–100%).

The NRLs' enumeration results and z-scores for the eight *Campylobacter*-positive samples included in PT 21 are presented in Table 4.

Table 3. Overall performance of the NRLs' enumeration of *Campylobacter* spp. (n=37) in proficiency test No. 21 (2018).

Grade	Scoring limits for each performance grade	Number (proportion) of NRLs with performance on all samples (n=10) within scores		Number (proportion) of NRLs with performance on *Campylobacter*-positive samples (n=8) within scores	
		All NRLs n=37	MS-NRLs n=32	All NRLs n=37	MS-NRLs n=32
Excellent	95.1–100%	20 (54%)	20 (63%)	20 (54%)	20 (63%)
Good	85.0–95.0%	11 (30%)	8 (25%)	10 (27%)	8 (25%)
Acceptable	70.0–84.9%	3 (8%)	3 (9%)	2 (5%)	1 (3%)
Needs improvement	57.0–69.9%	1 (3%)	0 (0%)	2 (5%)	2 (6%)
Poor	<57.0%	2 (5%)	1 (3%)	3 (8%)	1 (3%)

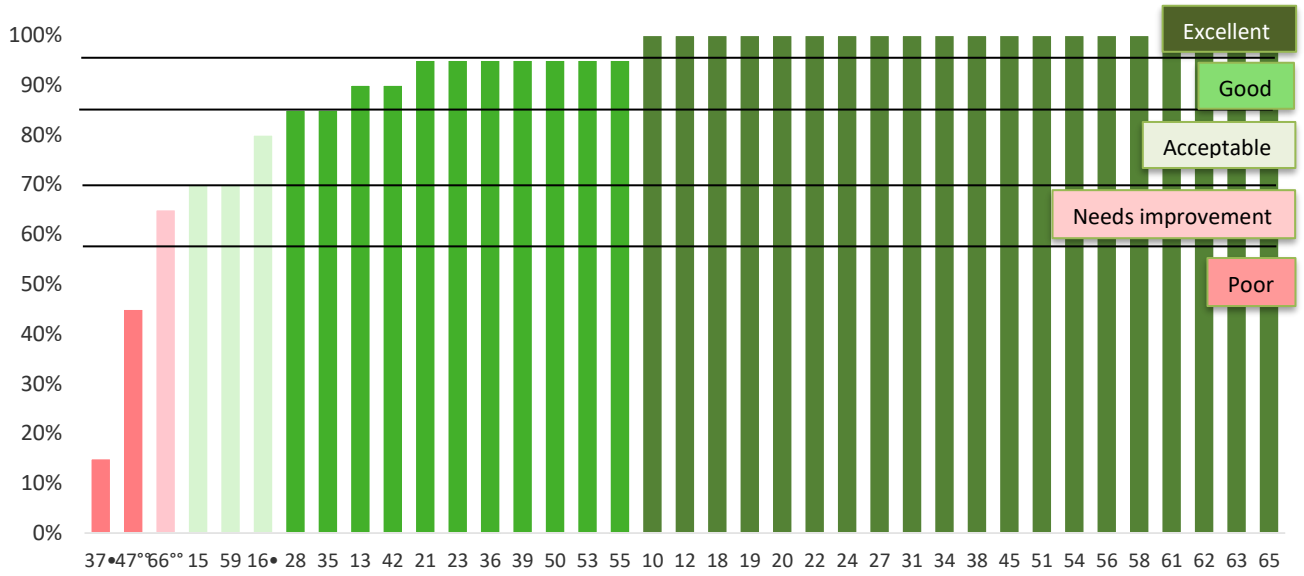

Figure 2. Distribution of the results of participating NRLs (n=37), represented by lab ID, in combined score for enumerations of the eight *Campylobacter*-positive samples and two *Campylobacter*-negative samples in PT 21 (2018). Limits for grading of the overall performance are marked by horizontal lines. Each ° stands for a false negative result, and • for a false positive result.

Table 4. Results from the enumeration and z-scores of *Campylobacter*-positive samples in proficiency test No. 21 (2018). Pale shadowed cells indicate values outside median values ±2σMAD, yellow for results below and green for results above the median value. Bright shadowed cells indicate values outside median values ±2.58σMAD and z-scores ±2 or lacking.

	Sample 2.		**Sample 3.**		**Sample 5.**		**Sample 6.**		**Sample 7.**		**Sample 8.**		**Sample 9.**		**Sample 10.**	
Lab id	log_{10} cfu/g	z-score	log_{10} cfu/g	z-score	log_{10} cfu/g	z-score	log_{10} cfu/g	z-score	log_{10} cfu/g	z-score	log_{10} cfu/g	z-score	log_{10} cfu/g	z-score	log_{10} cfu/g	z-score
10	3.71	-0.21	3.13	0.22	2.64	0.17	4.38	0.29	4.99	0.85	2.97	-0.34	3.84	0.35	1.86	-0.36
12	4.45	1.12	2.98	-0.12	2.86	0.51	4.53	0.49	5.37	1.27	3.07	-0.20	3.79	0.26	1.96	-0.04
13	4.54	1.29	3.24	0.47	2.52	-0.02	4.36	0.26	5.24	1.13	3.84	0.90	4.57	1.70	2.31	1.06
15	4.00	0.32	3.40	0.83	5.00	3.82	3.90	-0.35	2.40	-2.01	4.80	2.26	4.00	0.64	2.00	0.08
16	4.19	0.66	3.16	0.29	2.71	0.27	3.59	-0.76	3.92	-0.33	3.71	0.71	4.49	1.55	1.93	-0.14
18	3.91	0.15	3.28	0.56	2.79	0.40	4.41	0.33	5.13	1.01	3.44	0.33	3.53	-0.23	2.16	0.59
19	4.39	1.02	3.32	0.65	2.69	0.24	3.76	-0.54	3.60	-0.68	3.42	0.30	3.83	0.33	1.96	-0.04
20	3.70	-0.22	3.00	-0.08	2.20	-0.52	3.10	-1.41	3.80	-0.46	2.90	-0.44	3.60	-0.10	1.80	-0.55
21	2.98	-1.52	2.79	-0.55	1.77	-1.18	3.60	-0.75	3.75	-0.52	2.74	-0.67	3.56	-0.17	1.74	-0.74
22	3.60	-0.40	3.00	-0.08	2.10	-0.67	4.40	0.32	4.10	-0.13	3.50	0.41	3.80	0.27	2.10	0.40
23	3.64	-0.33	3.28	0.56	2.04	-0.76	2.58	-2.11	3.66	-0.62	3.04	-0.24	3.27	-0.71	2.19	0.68
24	3.04	-1.41	2.91	-0.28	1.95	-0.90	3.38	-1.04	3.89	-0.36	2.93	-0.40	3.72	0.13	1.81	-0.52
27	3.60	-0.40	2.80	-0.53	2.00	-0.83	4.30	0.18	3.80	-0.46	3.60	0.56	3.80	0.27	1.90	-0.23
28	3.19	-1.14	2.23	-1.82	2.17	-0.56	4.62	0.61	2.82	-1.54	2.50	-1.01	3.49	-0.30	1.69	-0.90
31	3.86	0.06	3.39	0.81	3.15	0.96	4.57	0.54	4.93	0.78	3.84	0.90	3.99	0.63	2.04	0.21
34	3.88	0.10	3.62	1.33	2.81	0.43	4.85	0.91	4.70	0.53	3.59	0.54	3.89	0.44	2.28	0.97
35	4.55	1.30	3.77	1.67	3.50	1.50	5.57	1.87	5.30	1.19	4.05	1.20	3.81	0.29	2.35	1.19
36	4.01	0.33	3.29	0.58	3.42	1.37	4.03	-0.18	4.78	0.62	4.05	1.20	3.93	0.51	2.19	0.68
37	2.20	-2.92	1.90	-2.56	1.45	-1.68	2.14	-2.69	1.58	-2.91	1.51	-2.41	1.65	-3.72	0.70	-4.03
38	3.90	0.14	3.18	0.33	2.30	-0.36	4.60	0.58	4.79	0.63	2.93	-0.40	3.32	-0.62	2.04	0.21
39	4.18	0.64	2.69	-0.78	1.48	-1.63	3.57	-0.79	4.72	0.55	3.20	-0.01	3.67	0.03	2.08	0.34
42	4.03	0.37	2.14	-2.02	2.31	-0.35	4.79	0.83	4.63	0.45	3.17	-0.05	3.91	0.48	1.91	-0.20
45	4.00	0.32	3.00	-0.08	2.50	-0.05	5.00	1.11	4.30	0.09	3.30	0.13	3.40	-0.47	2.30	1.03
47	<1.0	–	<1.0	–	2.02	-0.79	2.99	-1.57	2.31	-2.11	1.76	-2.06	2.44	-2.25	1.76	-0.69
50	2.85	-1.75	2.65	-0.87	1.70	-1.29	3.43	-0.97	4.73	0.56	2.60	-0.86	3.88	0.42	1.98	0.02
51	3.90	0.14	3.18	0.33	2.71	0.27	4.90	0.98	3.86	-0.40	3.18	-0.04	3.41	-0.45	2.11	0.43
53	3.00	-1.48	2.50	-1.21	2.20	-0.52	4.00	-0.22	4.30	0.09	3.20	-0.01	3.30	-0.65	1.50	-1.50
54	4.30	0.85	3.40	0.83	2.60	0.10	3.10	-1.41	4.70	0.53	3.40	0.27	3.80	0.27	2.30	1.03
55	4.00	0.32	2.90	-0.30	2.30	-0.36	4.40	0.32	4.20	-0.02	3.00	-0.30	3.30	-0.65	2.50	1.67
56	3.60	-0.40	3.10	0.15	2.40	-0.21	4.70	0.71	3.00	-1.35	3.40	0.27	3.90	0.46	1.90	-0.23
58	4.45	1.12	3.70	1.51	3.00	0.72	4.40	0.32	5.08	0.95	3.45	0.34	3.74	0.16	1.78	-0.61
59	4.92	1.98	3.89	1.93	2.43	-0.16	4.28	0.15	5.64	1.57	4.87	2.36	4.91	2.34	2.04	0.21
61	4.00	0.32	2.57	-1.05	2.92	0.60	5.18	1.35	4.73	0.56	3.20	-0.01	3.52	-0.25	1.90	-0.23
62	3.78	-0.08	2.69	-0.78	2.95	0.65	4.89	0.97	4.11	-0.12	3.00	-0.30	3.57	-0.15	1.81	-0.52
63	4.10	0.49	2.90	-0.30	2.21	-0.50	4.51	0.46	4.14	-0.09	2.65	-0.79	3.53	-0.23	1. 60*	-1.18
65	3.40	-0.76	3.20	0.38	2.80	0.41	4.90	0.98	4.70	0.53	3.30	0.13	3.90	0.46	2.10	0.40
66	<1.0	–	<1.0	–	3.11	0.90	4.32	0.21	4.41	0.22	1.60*	-2.28	3.08	-1.06	2.45	1.50
Median	3.90		3.10		2.50		4.38		4.30		3.20		3.74		1.98	
MAD	0.29		0.22		0.33		0.47		0.49		0.27		0.19		0.17	
σMAD	0.43		0.33		0.49		0.70		0.73		0.40		0.28		0.25	
Mean		3.82		3.03		2.53		4.16		4.22		3.21		3.65		1.97
SD		0.56		0.44		0.65		0.75		0.91		0.70		0.54		0.32

*reported as **present** but lower than this value, calculations based on this value

Detection and species identification of *Campylobacter* spp. (voluntary)

Detection and species identification of *Campylobacter* were voluntary parts of PT 21. Twenty-four (65%) of the 37 NRLs reported results of detection. Twenty-three NRLs used a procedure including enrichment, and four of them used direct plating as well. One laboratory did only direct plating. Of the NRLs that performed enrichment of the samples, thirteen used Bolton broth only, five Preston broth only, and five used both Bolton and Preston broth. All 24 NRLs did the plating on mCCD agar, and 19 NRLs on at least one additional medium: Preston agar (8), Skirrow agar (5), Butzler agar (3), CampyFood agar (2), CHROMagar (1), or CASA agar (1).

All 24 NRLs reported in their final answers growth of *Campylobacter* spp. in the eight positive samples and no growth of *Campylobacter* in the two negative samples. One NRL did not detect *Campylobacter* in sample No. 5 on mCCD agar, only on Preston agar. One NRL that reported false negatives on samples No. 2 and 3 in the enumeration part of the PT correctly detected *Campylobacter* in both these samples after enrichment.

Thirty-three (89%) NRLs reported results of species identification (Table 5). Seven NRLs identified species of *Campylobacter* from both direct cultured plates and from culture on selective media after enrichment. Fifteen NRLs did the species identification from direct cultured plates only and eleven NRLs from culture after enrichment only.

Table 5. Species identification reported by 33 NRLs in the voluntary part of proficiency test No. 21 (2018).

		Number of NRLs reporting				
Content of sample (vial)		*Campylobacter jejuni*	*Campylobacter coli*	*Campylobacter lari*	*Campylobacter* spp. but unable to identify species	Other/No growth
1.	Negative					33
2.	*Campylobacter lari*			32	1	
3.	*Campylobacter lari*			31	2	
4.	*Escherichia coli*		1			32
5.	*Campylobacter coli* & *Escherichia coli*		33			
6.	*Campylobacter jejuni*	33				
7.	*Campylobacter coli*	1	32			
8.	*Campylobacter jejuni*	33				
9.	*Campylobacter jejuni*	33				
10.	*Campylobacter jejuni*	33				

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, sensitivity to nalidixic acid and cephalotin, and H_2S production in triple sugar iron medium.

Seventeen of the 33 NRLs reported that they used MALDI-TOF MS for the species identification, in seven cases in combination with one or more other methods. Fifteen NRLs used one or more PCR assays, in eight 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). Thirteen NRLs used biochemicals methods (at least detection of catalase), in eight cases in combination with MALDI-TOF MS and/or PCR.

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

Good performance in detection of *Campylobacter*

Of the 24 NRLs reporting results for detection of *Campylobacter*, all NRLs fulfilled the criteria for excellent for detection of *Campylobacter*, and none scored below the acceptable criteria (Table 6). The overall median sensitivity in correctly detecting *Campylobacter* was 100% (50% CR: 100%–100%).

Table 6. Overall performance of NRLs sensitivity in correctly detecting *Campylobacter* in the voluntary part of proficiency test No. 21 (2018).

		Detection of *Campylobacter*	
Grade	**Sensitivity**	**Number of NRLs (%) All NRLs, n=24**	**Number of NRLs (%) MS-NRLs, n=22**
Excellent	95.1–100%	24 (100%)	22 (100%)
Good	85.0–95.0%	0 (0%)	0 (0%)
Acceptable	70.0–84.9%	0 (0%)	0 (0%)
Needs improvement	57.0–69.9%	0 (0%)	0 (0%)
Poor	<57.0%	0 (0%)	0 (0%)

Good performance in identification of *Campylobacter* spp.

Of the 33 NRLs reporting results for species identification of *Campylobacter*, 32 fulfilled the criteria for excellent or good performance for identification of *Campylobacter* spp., and none scored below the acceptable criteria (Table 7). The overall median sensitivity in correctly identifying *Campylobacter* spp. was 100% (50% CR: 100%–100%).

Table 7. Overall performance of NRLs sensitivity in correctly identifying *Campylobacter* spp. in the voluntary part of PT 21 (2018).

		Identification of *Campylobacter* spp.	
Grade	**Sensitivity**	**Number of NRLs (%) All NRLs, n=33**	**Number of NRLs (%) MS-NRLs, n=29**
Excellent	95.1–100%	29 (88%)	25 (86%)
Good	85.0–95.0%	3 (9%)	3 (10%)
Acceptable	70.0–84.9%	1 (3%)	1 (3%)
Needs improvement	57.0–69.9%	0 (0%)	0 (0%)
Poor	<57.0%	0 (0%)	0 (0%)

Summary of the proficiency test number 21, 2018

Of the 37 laboratories 84% of the NRLs had good or excellent performance considering the enumeration which is about the same level as the three previous years (Table 8).

Table 8. Overall performance of the NRLs' enumeration of *Campylobacter* spp. in proficiency test (PT) No. 21, 2018, compared to performance in PTs for previous years, as well as grades for the results of the NRLs.

	All samples (n=10)	Only *Campylobacter*-positive samples (n=8)			
Grade	**PT 21 (2018) Number of NRLs (%) n=37**	**PT 21 (2018) Number of NRLs (%) n=37**	**PT 19 (2017) Number of NRLs (%) n=36**	**PT 17 (2016) Number of NRLs (%) n=36**	**PT 15 (2015) Number of NRLs (%) n=36**
Excellent	20 (54%)	20 (54%)	22 (61%)	26 (72%)	17 (47%)
Good	11 (30%)	10 (27%)	9 (25%)	6 (17%)	12 (33%)
Acceptable	3 (8%)	2 (5%)	2 (6%)	1 (3%)	2 (6%)
Needs improvement	1 (3%)	2 (5%)	0 (0%)	2 (6%)	2 (6%)
Poor	2 (5%)	3 (8%)	3 (8%)	1 (3%)	3 (8%)

Detection and identification of *Campylobacter* spp. was voluntary in PT 21, still 24 (65%) and 33 (89%) of the 37 laboratories reported results of detection and species identification, respectively (Table 9 and 10). The performance was high (100% and 97% excellent or good) both for detection and identification.

Table 9. Overall performance of NRLs' sensitivity in correct detection of *Campylobacter* spp. in proficiency test No. 21, 2018, compared to performance in proficiency tests (PT) for previous years, as well as grades for the results of the NRLs.

Grade	**PT 21 (2018) Number of NRLs (%) n=24**	**PT 19 (2017) Number of NRLs (%) n=24**	**PT 17 (2016) Number of NRLs (%) n=29**	**PT 15 (2015) Number of NRLs (%) n=27**
Excellent	24 (100%)	24 (100%)	27 (93%)	26 (96%)
Good	0 (0%)	0 (0%)	1 (3%)	0 (0%)
Acceptable	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Needs improvement	0 (0%)	0 (0%)	1 (3%)	0 (0%)
Poor	0 (0%)	0 (0%)	0 (0%)	1 (4%)

Table 10. Overall performance of NRLs' sensitivity in correct species identification of *Campylobacter* in proficiency test No. 21, 2018, compared to performance in proficiency tests (PT) for previous years, as well as grades for the results of the NRLs.

Grade	PT 21 (2018) Number of NRLs (%) n=33	PT 19 (2017) Number of NRLs (%) n=31	PT 17 (2016) Number of NRLs (%) n=29	PT 15 (2015) Number of NRLs (%) n=27
Excellent	29 (88%)	30 (97%)	27 (93%)	26 (96%)
Good	3 (9%)	1 (3%)	1 (3%)	0 (0%)
Acceptable	1 (3%)	0 (0%)	0 (0%)	0 (0%)
Needs improvement	0 (0%)	0 (0%)	1 (3%)	0 (0%)
Poor	0 (0%)	0 (0%)	0 (0%)	1 (4%)

The majority of the NRLs had excellent or good performance in all parts: enumeration, detection, and species identification, meeting the requirements of being a NRL. Two NRLs (one MS-NRL) need to improve their performance. The EURL-*Campylobacter* has offered assistance to the MS-NRL with poor performance.

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