EURL-*CAMPYLOBACTER*

REPORT

PROFICIENCY TEST NUMBER 30

Detection and species identification of *Campylobacter* spp.

Publication history

Version	Date
Final version	2021-12-31

Contents

Abbreviations

C.	*Campylobacter*
cfu	colony forming units
EU	European Union
EURL	European Union reference laboratory
FP	false positive
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
NRL	national reference laboratory (in this report also used for a laboratory with a similar function in a non-EU Member State)
PCR	polymerase chain reaction
PT	proficiency test
SD	standard deviation
spp.	species

Summary of proficiency test number 30, 2021

The EU reference laboratory for *Campylobacter* organised proficiency test (PT) number 30 on detection and species identification of *Campylobacter* in March 2021. The PT included detection and species identification of *Campylobacter* spp. in 18 samples of raw milk with vials with or without freeze-dried *Campylobacter*. The matrix could be considered both as a food sample and an animal sample. The objective was to assess the performance of the national reference laboratories (NRLs) to detect and identify *Campylobacter* species in raw milk.

Thirty-six NRLs in 27 EU Member States (some Member States have more than one NRL) and in Iceland, Norway, Switzerland, and United Kingdom participated in the PT.

Of the 36 NRLs, 33 followed ISO 10272-1:2017 for detection of *Campylobacter* spp., and three NRLs used other methods. All 36 NRLs used an enrichment protocol, and 33 NRLs used the recommended procedure A with enrichment in Bolton broth. Twelve NRLs used more than one procedure.

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. Twenty-four NRLs (67%) fulfilled the criteria for excellent or good performance in detection of *Campylobacter*, and three (two Member State NRLs, MS-NRLs) scored below the acceptable limit. Two NRLs scored below the limit because of low sensitivity, and one NRL because of low specificity.

Of the 35 NRLs reporting results for species identification, 34 fulfilled the criterion for excellent performance in identification of *Campylobacter* spp., and none scored below the acceptable limit.

In summary, the majority of the NRLs met the criteria for excellent or good performance in both detection and species identification, and only three NRLs scored below the acceptable limit in detection. One MS-NRL with poor performance because of low sensitivity in detection has been offered and performed an extra PT.

Introduction

The proficiency test (PT) number 30 on detection and species identification of *Campylobacter* was organised by the EU reference laboratory (EURL) for *Campylobacter* in March 2021. Participation in the PT was mandatory for at least one national reference laboratory (NRL) in each Member State. Thirty-six NRLs in 27 EU Member States (some Member States have more than one NRL) and in Iceland, Norway, Switzerland, and United Kingdom participated in the PT. The test results and operational details were reported to the EURL from all 36 NRLs. Thirty-four NRLs reported that they were accredited for detection of *Campylobacter* and 25 were also accredited for enumeration of *Campylobacter*.

The PT included detection and species identification of *Campylobacter* spp. in 18 samples of raw milk 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 raw milk.

Table 1. Bacteria in the vials in proficiency test No. 30 (2021).

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)	SD [c] (log_{10} cfu)
11	–						
12	*Campylobacter lari*	SLV300	2.81	2.47	(low)		0.08
13	*Campylobacter jejuni* [d]	SVA025	3.20	2.86	(low)		0.11
14	*Campylobacter jejuni* [d]	SVA059	4.53	4.19	(high)		0.09
15	*Escherichia coli*	SVA045				4.74	0.08
16	–						
17	*Campylobacter coli*	SVA060	4.45	4.11	(high)		0.09
18	*Campylobacter lari*	SVA050	3.95	3.61	(high)		0.06
19	*Campylobacter lari*	SVA048	4.22	3.88	(high)		0.10
20	*Campylobacter lari*	SVA054	3.14	2.80	(low)		0.06
21	*Campylobacter jejuni* [d]	SVA059	4.53	4.19	(high)		0.09
22	*Campylobacter coli*	SVA051	3.19	2.85	(low)		0.12
23	*Campylobacter jejuni* [d]	SVA055	3.28	2.94	(low)		0.10
24	*Escherichia coli*	SVA045				4.74	0.08
25	*Escherichia coli*	SVA045				4.74	0.08
26	*Campylobacter coli*	SVA060	4.45	4.11	(high)		0.09
27	–						
28	*Campylobacter coli*	SVA051	3.19	2.85	(low)		0.12

[a] Total quantity of *Campylobacter* in each vial and per test portion of 10 ml, after mixing with raw milk to a total volume of 22 ml. The low and high levels were based on a LOD_{50} of 57 cfu per test portion of 10 ml raw milk, according to ISO 10272-1:2017. The theoretical content per test portion varied from 5 to 15 × LOD_{50} in low-level samples and was at least 70 × LOD_{50} in high-level samples.

[b] Total quantity of *Escherichia coli* in each vial.

[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) *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 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 samples of raw milk, which could be considered both as food samples and animal samples. The participants were instructed to divide the milk into 18 portions and mix the content of the vials with the milk, making up a volume of 22 ml for each sample. 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 5 and 15 times a LOD_{50} of 57 cfu (according to ISO 10272-1:2017, annex C) and in high-level samples between 70 and 270 times LOD_{50}.

Preparation of the raw milk

The raw milk used as matrix in the PT was obtained directly from a local milk farm two months before distribution of the PT and was tested negative for presence of *Campylobacter* spp. The milk contained an abundant background flora with several genera and species, according to the cultivation of milk (before and after freezing) on blood agar and typing of selected colonies with matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS): *Staphylococcus* spp., *Streptococcus* spp. (including *Streptococcus dysgalactiae*), *Bacillus* spp., *Citrobacter braakii*, and coliform bacteria. The milk was aliquoted and freeze-stored until dispatch.

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 or the Swedish Food Agency.

Each combination of vial and matrix was prepared and tested by the EURL according to ISO 10272-1:2017, procedure A (enrichment in Bolton broth) and procedure B (enrichment in Preston broth), at least three times: before dispatch, just after dispatch, and one week after dispatch, i.e. after the last time for start of the analysis by the participants.

Distribution of the proficiency test

The PT samples were distributed from the EURL on the 8th of March, 2021. The samples were placed in foam boxes along with freezing blocks. The foam 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 a plastic bottle with about 420 ml raw milk. A Micro-T-Log was included in longer shipments to record the temperature every second hour during transport.

Of the 36 participating NRLs, 31 received the test one day after dispatch and the remaining 5 received it two days after dispatch.

The PT analyses were recommended to be started as soon as possible after the arrival and at the latest on the 12th of March, 2021. All results had to be reported in the Questback Essentials system by the 19th of April, 2021. 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, samples with raw milk and vials were recommended to be stored at cold temperature (between 1 °C and 8 °C). If the analysis was started more than 24 hours after the arrival, the vials were recommended be stored at −20 °C or colder.

Methods for analysis

The NRLs were recommended to follow ISO 10272-1:2017, procedure A (enrichment in Bolton broth) for performing the PT but were allowed to use another method if their standard laboratory procedure followed a different method. The amount of milk provided allowed the laboratories to voluntarily perform enrichment in two separate broths, e.g. Bolton broth and Preston broth, 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 36 participating NRLs, 22 reported using gas-generating kits, nine microaerobic incubators, seven the Anoxomat® system and one another method (zip-lock bags filled with gas). 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 2. The cut-off for good **performance in identification** of *Campylobacter* species was set to 85.0%.

Table 2. The minimum number of correct results (*Campylobacter* detected or not detected) needed for each combined performance grade, and the corresponding measures of sensitivity (Se), accuracy (Acc) and specificity (Sp), in proficiency test No. 30 (2021). 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)**	**Neg (n = 6)**	**Se low**	**Se high**	**Se total**	**Acc**	**Sp**
Excellent	5	6	6	83%	100%	92%	94%	100%
Good	4	5	6	67%	83%	75%	83%	100%
Acceptable	3	4	5	50%	67%	59%	67%	83%
Needs improvement	2	3	4	33%	50%	42%	50%	67%

Results

Detection and species identification of *Campylobacter*

Proficiency test number 30 was distributed to 36 NRLs and all of them 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. Four NRLs started the analysis the day after the samples were dispatched from the EURL, 21 NRLs two days after, four NRLs three days after, six NRLs four days after, and one NRL seven days after.

Of the 36 NRLs, 33 followed ISO 10272-1:2017 for detection of *Campylobacter* spp., and three NRLs used other culture methods. All 36 NRLs used an enrichment protocol: 33 NRLs with enrichment in Bolton broth (the recommended procedure A), 12 NRLs with enrichment in Preston broth, and one NRL with enrichment in CampyFood® broth. Six NRLs also performed direct plating. Twelve NRLs used more than one procedure.

Four NRLs reported to have used various polymerase chain reaction (PCR) assays as additional detection procedures, either in all samples or in culture-negative samples. One NRL performed real-time PCR (Josefsen et al. 2010, Josefsen et al. 2004, Lund et al. 2004) after enrichment, but did not specify if any deviating results were obtained compared with plating. Other PCR assays used for detection were those published by Denis et al. (1999), Wang et al. (2002), Vandamme et al. (1997), and Toplak et al. (2012). Two NRLs reported their final results based on the culture method only, but also to have detected *C. coli* with PCR in one or two culture-negative samples (Table 3, footnote c and d). One NRL reported *C. coli* detected with conventional PCR in sample No. 16 and No. 28 as their final results (Table 3, footnote b).

Thirty-five NRLs did the plating on modified charcoal cefoperazone deoxycholate (mCCD) agar, and 29 plated on at least one additional medium. Other media used for plating were Preston agar (10), CampyFood® agar (5), Skirrow agar (5), Butzler agar (5), Karmali agar (3), CASA® agar (2), CHROMagar™ Campylobacter (2), Brilliance™ CampyCount agar (1), RAPID Campylo BioRad agar (1), and Abeyta Hunt Bark agar (1).

The presumtive *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 MALDI-TOF MS or PCR. Twenty-six of the 36 NRLs used microscopic examination as part of the confirmation procedure. Twenty-four NRLs used oxidase test, in 20 cases in combination with aerobic growth at 25 °C, and in 17 cases in combination with MALDI-TOF and/or PCR. One NRL used latex agglutination in addition to oxidase test and aerobic growth at 25 °C. Eighteen NRLs used MALDI-TOF MS for confirmation, in 10 cases in combination with other techniques other than microscopic examination. Thirteen NRLs used one or more PCR assays, in all cases in combination with other techniques. Six 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 nalidixic acid and cephalotin, hydrogen sulphide production in triple sugar iron medium, nitrate reduction, growth in 3.5% sodium chloride or 1% glycine, and growth on MacConkey or nutrient agar. One NRL reported to also have performed tests of growth at different temperatures.

Nineteen of the 36 NRLs used MALDI-TOF MS for the species identification, in seven cases in combination with other techniques. Eighteen NRLs used one or more PCR assays, in 15 cases in combination with other techniques. Five NRLs reported to have used the multiplex PCR assay published by Wang et al. (2002). Seventeen NRLs used biochemicals tests (at least detection of catalase), in twelve cases in combination with MALDI-TOF MS and/or PCR. One NRL did not perform the species identification because they normally send strains to another laboratory for typing.

Seventeen NRLs used one technique only (a set of biochemical tests and/or tests of growth regarded as one technique), 17 NRLs combined two techniques, and one NRL used three techniques for the species identification.

Of the 36 NRLs, seven reported correct results of detection, i.e. correct identification of the 12 samples with *Campylobacter* and the six samples without *Campylobacter* (Figure 1). Six false positive results, whereof one obtained with PCR only, were reported by five NRLs. Six of the 36 NRLs reported correct species in all 12 samples that had been inoculated with *Campylobacter* spp., and 34 NRLs correct species in all inoculated samples where *Campylobacter* spp. had been detected.

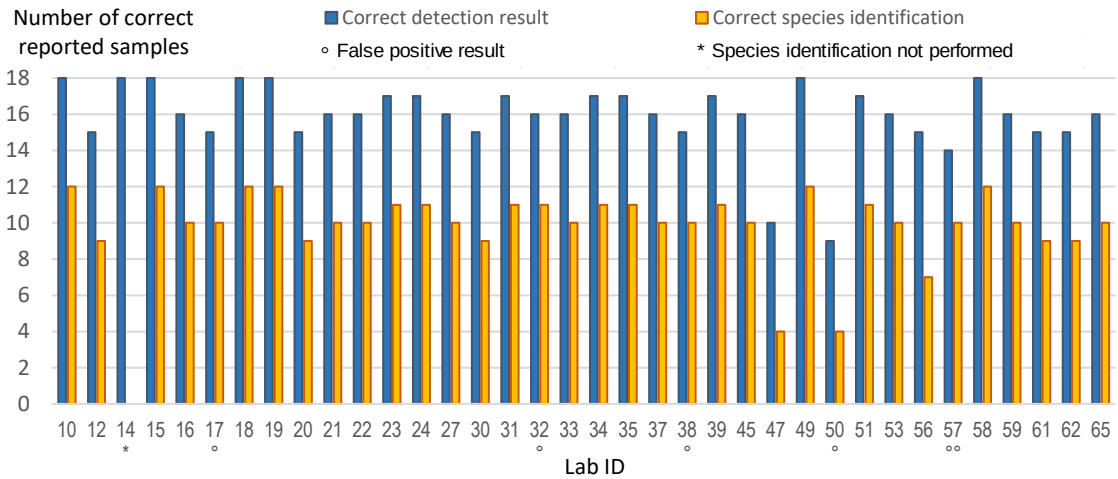

Figure 1. Distribution of correct results by 36 NRLs participating in proficiency test No. 30 (2021) in the detection and species identification of *Campylobacter* spp. in raw milk.

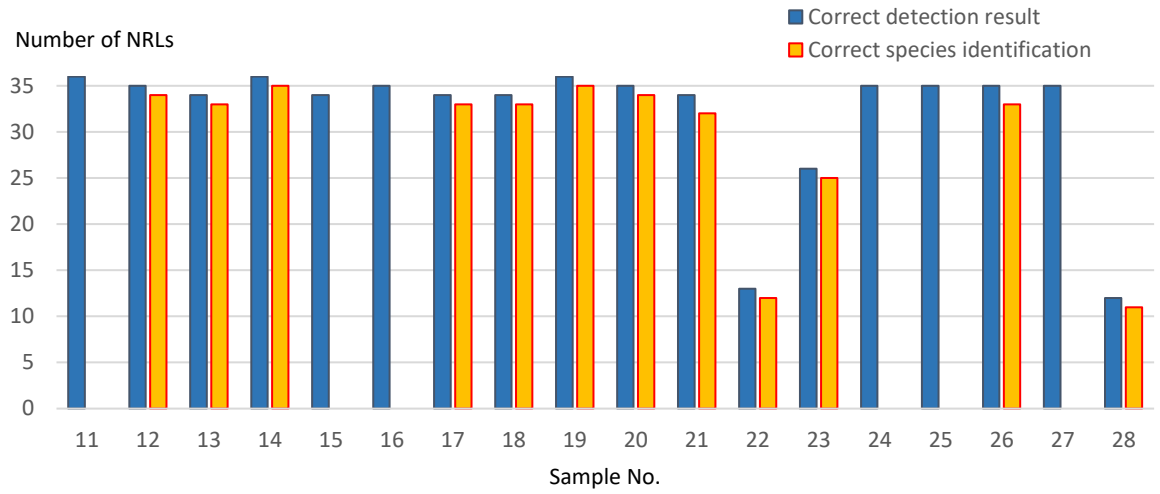

Figure 2. Number of NRLs participating in proficiency test No. 30 (2021) that correctly reported results in the detection and species identification of *Campylobacter* in 18 samples of raw milk. In total, 36 NRLs performed detection and 35 NRLs species identification.

All 36 NRLs reported correct results of detection for three (two samples with and one sample without *Campylobacter*) of the 18 samples (Figure 2, Table 3). The two samples containing *Campylobacter* correctly detected by all NRLs (No. 14 and 19) were also correctly identified by all 35 NRLs performing species identification, as *C. jejuni* and *C. lari*. Samples No. 22 and 28 (*C. coli*) had a detection rate of 34.7%, and sample No. 23 (*C. jejuni*) a detection rate of 72.2%. Failure to detect *Campylobacter* in any of these three low-level samples was probably in some cases caused by chance alone. This was taken into account when evaluating the NRLs' performance for the low-level samples.

Generally, failure in detection of *Campylobacter* in low-level samples were more frequent than expected, based on the theoretical levels in the freeze-dried vials and the pre-tests with raw milk performed by the EURL. This however correlated with the tests performed by the EURL after the test had been subjected to transport conditions, which implies instability of the levels during transport. Despite this, the detection rates were still in line with the recommendations for low-level samples in detection PTs according to ISO 22117:2019, and the results were thus judged usable for evaluation of the NRLs' performance, after adjustment of the grading scale.

Table 3. Results of detection and species identification in 18 samples of raw milk in proficiency test No. 30 (2021). In total 35 NRLs performed species identification.

Sample No.	Bacterial species in vial	*C. jejuni*	*C. coli*	*C. lari*	*Campylobacter* spp. but unable to identify species	Growth of other, not *Campylobacter*	No growth at all
11	–					2	34
12	*Campylobacter lari*			34	1[a]	1	
13	*Campylobacter jejuni*	33			1[a]	2	
14	*Campylobacter jejuni*	35			1[a]		
15	*Escherichia coli*	1	1			31	3
16	–		1[b]			3	32
17	*Campylobacter coli*		33		1[a]	2	
18	*Campylobacter lari*			33	1[a]	1	1
19	*Campylobacter lari*			35	1[a]		
20	*Campylobacter lari*			34	1[a]	1	
21	*Campylobacter jejuni*	32		1	1[a]	1	1
22	*Campylobacter coli*		12		1[a]	2	21[c]
23	*Campylobacter jejuni*	25			1[a]	2	8
24	*Escherichia coli*		1			32	3
25	*Escherichia coli*		1			32	3
26	*Campylobacter coli*	1	33		1[a]	1	
27	–		1			5	30
28	*Campylobacter coli*		11[b]		1[a]	2	22[d]

[a] All answers "*Campylobacter* spp. but unable to identify species" were from the same NRL, which reported to not have performed the species identification.

[b] One NRL reported to have detected *C. coli* by conventional PCR.

[c] Two NRLs reported to have detected *C. coli* by conventional PCR (Denis et al. 1999) or qPCR (Toplak et al. 2012).

[d] One NRL reported to have detected *C. coli* by conventional PCR (Denis et al. 1999).

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

Of the 36 participating NRLs, 24 NRLs (21 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 4). Two NRLs scored below the limit because of low sensitivity, and one because of low specificity (two false positive samples out of six negative samples). Thirty-four NRLs (all 30 MS-NRLs) fulfilled the criterion for excellent performance in identification of *Campylobacter* spp., and none scored below the acceptable limit (Table 5).

Table 4. Combined performance grades in detection of *Campylobacter* spp. in proficiency test No. 30 (2021).

Combined performance in detection of *Campylobacter* spp.		
Grade	**Number of NRLs (%) All NRLs, n=36**	**Number of NRLs (%) MS-NRLs, n=30**
Excellent	14 (39%)	12 (40%)
Good	10 (28%)	9 (30%)
Acceptable	9 (25%)	7 (23%)
Needs improvement	1 (3%)	1 (3%)
Poor	2 (6%)	1 (3%)

Table 5. Overall performance of NRLs' sensitivity in correct species identification of *Campylobacter* in proficiency test No. 30 (2021).

Performance in identification of *Campylobacter* spp.			
Grade	**Sensitivity**	**Number of NRLs (%) All NRLs, n=35**	**Number of NRLs (%) MS-NRLs, n=30**
Excellent	95.1–100%	34 (97%)	30 (100%)
Good	85.0–95.0%	0 (0%)	0 (0%)
Acceptable	70.0–84.9%	1 (3%)	0 (0%)
Needs improvement	57.0–69.9%	0 (0%)	0 (0%)
Poor	<57.0%	0 (0%)	0 (0%)

All performance parameters for detection and identification of *Campylobacter* spp. in raw milk for all participants are presented in Table 6.

Enrichment in Preston broth, in addition to or instead of the recommended enrichment in Bolton broth, did not affect the detection ability or overall performance of NRLs. There was at least a tendency for NRLs using more than one selective medium for plating to have a higher recovery rate in the low-level samples. Among the 29 NRLs that used two or more agar plates, 23 (79%) detected at least 4/6 low-level samples, and 13 (45%) detected at least 5/6 low-level samples (qualifying for the *Good* and *Excellent* grade, respectively). The same numbers for the seven NRLs that used one agar plate only was five (71%) NRLs detecting at least 4/6 and two (29%) NRLs detecting at least 5/6 low-level samples. However, although it seems logical that streaking a sample on more plates leads to a higher probability of detecting *Campylobacter* at a low level, the groups using different numbers of plates (and even more different agars) were small and do not allow any definitive conclusion to be drawn regarding this.

Table 6. 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 36 NRLs participating in proficiency test No. 30 (2021). 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
10	100%	100%	100%	100%	100%	Excellent	100%
12	50%	100%	75%	100%	83%	Acceptable	100%
14	100%	100%	100%	100%	100%	Excellent	–
15	100%	100%	100%	100%	100%	Excellent	100%
16	67%	100%	83%	100%	89%	Good	100%
17	67%	100%	83%	83%	83%	Acceptable	100%
18	100%	100%	100%	100%	100%	Excellent	100%
19	100%	100%	100%	100%	100%	Excellent	100%
20	50%	100%	75%	100%	83%	Acceptable	100%
21	67%	100%	83%	100%	89%	Good	100%
22	67%	100%	83%	100%	89%	Good	100%
23	83%	100%	92%	100%	94%	Excellent	100%
24	83%	100%	92%	100%	94%	Excellent	100%
27	67%	100%	83%	100%	89%	Good	100%
30	50%	100%	75%	100%	83%	Acceptable	100%
31	83%	100%	92%	100%	94%	Excellent	100%
32	83%	100%	92%	83%	89%	Acceptable	100%
33	67%	100%	83%	100%	89%	Good	100%
34	83%	100%	92%	100%	94%	Excellent	100%
35	83%	100%	92%	100%	94%	Excellent	100%
37	67%	100%	83%	100%	89%	Good	100%
38	67%	100%	83%	83%	83%	Acceptable	100%
39	83%	100%	92%	100%	94%	Excellent	100%
45	67%	100%	83%	100%	89%	Good	100%
47	17%	50%	33%	100%	56%	Poor	100%
49	100%	100%	100%	100%	100%	Excellent	100%
50	33%	33%	33%	83%	50%	Poor	100%
51	83%	100%	92%	100%	94%	Excellent	100%
53	67%	100%	83%	100%	89%	Good	100%
56	50%	100%	75%	100%	83%	Acceptable	78%
57	67%	100%	83%	67%	78%	Needs improvement	100%
58	100%	100%	100%	100%	100%	Excellent	100%
59	67%	100%	83%	100%	89%	Good	100%
61	50%	100%	75%	100%	83%	Acceptable	100%
62	50%	100%	75%	100%	83%	Acceptable	100%
65	67%	100%	83%	100%	89%	Good	100%

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