EURL-*CAMPYLOBACTER*

REPORT

PROFICIENCY TEST NUMBER 31

Enumeration (and voluntary species identification) of

Campylobacter

Publication history

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Abbreviations

C.	*Campylobacter*
cfu	colony forming units
CR	central range
EU	European Union
EURL	European Union reference laboratory
ISO	International Organization for Standardization
$\log_{10}$	logarithm to base 10 (common logarithm)
MADe	scaled median absolute deviation
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 used for all participating laboratories, also in non-EU Member States)
PCR	polymerase chain reaction
PT	proficiency test
spp.	species

Summary of the proficiency test number 31, 2022

The EU reference laboratory for *Campylobacter* organised proficiency test (PT) number 31 on enumeration of *Campylobacter* spp. in chicken skin in March 2022. The PT included enumeration of *Campylobacter* spp. in 10 samples of chicken skin mixed with vials with or without freeze-dried *Campylobacter*. The objective was to assess the performance of the national reference laboratories (NRLs) to enumerate *Campylobacter* in chicken skin. Species identification of detected *Campylobacter* was included as a voluntary part of PT 31.

Participation in PT 31 was mandatory for at least one NRL per MS. Thirty-five NRLs in 27 EU Member States (some Member States have more than one NRL) and in Albania, Iceland, Norway, and United Kingdom received the PT and responses were reported from 34 NRLs. Thirty-two NRLs reported to have followed the recommended method of ISO 10272-2:2017, and two NRLs used other methods.

Thirty (88 %) NRLs fulfilled the criterion for excellent or good performance in enumeration of *Campylobacter* spp. No NRL scored below the acceptable limit, but one NRL failed to report final results.

Thirty of the 34 NRLs reported results of species identification of *Campylobacter*, and 28 (93 %) of them fulfilled the criterion for excellent or good performance in identification of *Campylobacter* spp. No NRL scored below the acceptable limit. Four misidentifications of species were reported.

In summary, the majority of the NRLs met the criteria for excellent or good performance in both enumeration and species identification, and no NRL scored below the acceptable limit in enumeration. The NRL that failed to report final results has been offered and performed an extra PT.

Introduction

Proficiency test (PT) number 31 on enumeration of *Campylobacter* spp. in chicken skin was organised by the EU reference laboratory (EURL) for *Campylobacter* in March 2022. Thirty-five national reference laboratories (NRLs) in 27 EU Member States (some Member States have more than one NRL) and in Albania, Iceland, Norway, and United Kingdom received the PT. The test results and operational details were reported to the EURL from 34 NRLs.

Thirty NRLs reported that they were accredited for detection of *Campylobacter* and 25 that they were accredited for enumeration of *Campylobacter*. Five NRLs were accredited for detection only, and one NRL reported that the accreditation currently was suspended for both enumeration and detection.

The PT included enumeration of *Campylobacter* spp. in 10 samples of chicken skin mixed with vials with or without freeze-dried *Campylobacter* (Table 1). The objective was to assess the performance of the NRLs to enumerate *Campylobacter* spp. in chicken skin. Species identification of detected *Campylobacter* was included as a voluntary part of PT 31.

Table 1. Contents of the 10 vials distributed to the NRLs in proficiency test No. 31 (2022).

Sample No.	Species	Level [b] (log_{10} cfu/vial)	Standard deviation [b] (log_{10} cfu)	Batch No.
1	*Campylobacter coli*	4.71	0.12	SLV367
2	*Campylobacter coli*	3.76	0.09	SLV334
3	*Campylobacter coli*	4.71	0.12	SLV367
4	*Campylobacter jejuni* [a]	4.64	0.03	SLV336
5	*Campylobacter jejuni* [a]	4.11	0.03	SLV313
	+ Escherichia coli	3.56	0.12	
6	*Campylobacter lari*	2.81	0.15	SLV297
7	*Campylobacter lari*	5.15	0.07	SLV335
8	*Escherichia coli*	4.19	0.04	SLV369
9	*Campylobacter jejuni* [a]	4.11	0.03	SLV313
	+ Escherichia coli	3.56	0.12	
10	Negative			SLV337

[a] The *C. jejuni* strains were hippurate positive.

[b] According to homogeneity test of 10 vials after the production. The maximum standard deviation allowed was 0.15 log_{10} cfu.

Terms and definitions

- *Campylobacter* spp.: Thermotolerant *Campylobacter* spp., i.e. which are able to grow at 41.5 °C, foremost (but not exclusively) *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari* and *Campylobacter upsaliensis*.
- Enumeration of *Campylobacter*: Determination of the number of *Campylobacter* colony forming units (cfu) per g.
- Confirmation of *Campylobacter* spp.: Microorganisms suspected to be *Campylobacter* spp. are confirmed as such by biochemical tests and/or molecular methods.
- Species identification of *Campylobacter*: Identification of thermotolerant *Campylobacter* species with biochemical tests and/or 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 two years. The broilers were slaughtered at a slaughterhouse with a history of low level of *Campylobacter*-positive flocks (0.4 % during 2020).

The chicken thigh skin was tested on arrival in triplicate with enrichment in both Bolton and Preston broth and by direct streak from each initial suspension on modified charcoal cefoperazone deoxycholate (mCCD) agar. The chicken skin tested negative for presence of *Campylobacter* as well as background flora. In addition, caecal samples from the chicken flock tested negative for *Campylobacter*. The chicken skin was cut in smaller pieces, divided up into portions of about 120 g each and 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 by the Swedish Food Agency and tested for stability and homogeneity by the producer. Before choosing the vials for the PT, the EURL tested three vials of each batch with mCCD agar to ensure expected levels and functionality.

To test for stability during transport conditions, the EURL performed enumeration of *Campylobacter* spp. in chicken skin (of the batch prepared for the PT) according to ISO 10272-2:2017 on six occasions (see Table 2). These tests were performed before dispatch on vials stored in “best case” transport conditions (5 °C for 24 h) and “worst case” transport conditions (5 °C for 24 h, 15 °C for 24 h, and 5 °C for 24 h). They were also performed one day after dispatch (“best case” conditions), one week after dispatch (“worst case” conditions) and two weeks after dispatch, at the last date for start of analysis by the participants (both “best case” and “worst case” conditions).

The levels of *Campylobacter* in vials stored in "worst case" conditions were similar (both higher and lower) to those stored in "best case" conditions, and the small variation observed was accounted the variability of each vial and technical variation of the method. The PT was concluded to be stable.

Table 2. Outline of stability testing under transport conditions for proficiency test No. 31 (2022).

Test occasion	Storage condition [a]	Number of samples tested
Before dispatch	Best case	Each vial with *Campylobacter* × 2
Before dispatch	Worst case	Each vial with *Campylobacter* × 3
Day after dispatch	Best case	The complete test
One week after dispatch	Worst case	Each vial with *Campylobacter* × 2
Two weeks after dispatch	Best case	The complete test
Two weeks after dispatch	Worst case	Each vial with *Campylobacter* × 2

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

Distribution of the proficiency test

The PT samples were distributed from the EURL on the 7th of March, 2022. 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 10 numbered vials, each containing freeze-dried material with or without *Campylobacter* spp., and one plastic bag with about 120 g of frozen chicken skin. The skin was to be divided into 10 g portions, one for each of the 10 vials. A Micro-T-Log was included in each package to record the temperature every second hour during transport.

Twenty-nine NRLs received the PT within one day after the packages had been dispatched from the EURL, and five NRLs within two days. Due to logistic transport issues, a second distribution from the EURL was done on the 21st of March, 2022. The NRL received the test one day after this second dispatch (Table 3).

The analysis was recommended to be started the same week as the PTs were dispatched from the EURL, and at the latest on the 21st of March. Instructions for preparation of an initial dilution of each sample were included in the packages and were also sent out by e-mail a few days before the PT distribution. The chicken skin was recommended to be stored at − 20 °C and the vials at −20 °C or −70 °C until start of analysis. The dates for start of analysis are presented in Table 3.

Table 3. Dates of arrival and start of analysis of proficiency test No. 31, 2022.

Arrival	**Number of NRLs n=35** [a]	**Start of analysis**	**Number of NRLs n=34**
8th of March	29	8th of March	1
9th of March	5	9th of March	9
22nd of March [b]	1	10th of March	4
		11th of March	1
		14th of March	6
		15th of March	6
		16th of March	2
		21st of March	4
		23rd of March [b]	1

[a] One NRL received the test but did not report final results.

[b] One NRL received a new package after second dispatch 21st of March.

Methods for analysis

The NRLs were recommended to follow ISO 10272-2:2017 for performing PT 31. However, if their standard laboratory procedure followed a different method, they were allowed to use that method for the test. Thirty-two NRLs reported to have followed the recommended method of ISO 10272-2:2017, and two NRLs used other methods (NMKL 119 3rd ed., 2007, and an internal method, respectively).

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 34 NRLs, 20 reported using commercial gas-generating kits, nine microaerobic incubators, five the Anoxomat® system and two other methods (zip-lock bags filled with gas or microaerophilic gas generating jars). Some of the NRLs used more than one system.

Assessing the performance of the NRLs

Assessment of performance in enumeration

The median values of the log-transformed cfu of *Campylobacter* spp. reported by all NRLs were used as assigned values for the eight samples positive for *Campylobacter*. The performance in enumeration was assessed by using scaled median absolute deviation (MADe) from the median values for calculating z-scores. The scaled MADe method is used to identify outlying counts when fewer than 50 participants undertake an enumeration (ISO 22117:2019).

A scoring system was used for assessing the performance in enumeration of each *Campylobacter*-positive sample, where results within median value ± 2σMADe ($|z| \leq 2.0$) were given score 2, results between ± 2σMADe and ± 3σMADe ($2.0 < |z| \leq 3.0$) were given score 1 and results outside ± 3σMADe ($|z| > 3.0$) were given score 0. For the four samples with the most homogeneous results (sample No. 1, 3, 5, and 9), σMADe was adjusted to 0.25 $\log_{10}$ cfu/g. By this adjustment, a result within 0.5 $\log_{10}$ units of the participants' median value was determined to be acceptable (given the maximum score 2), according to the 0.5 $\log_{10}$ rule (ISO 22117:2019). For the samples without *Campylobacter* a score of 2 was given when no *Campylobacter* spp. were reported, and a score of 0 when a false positive result was reported.

For sample No. 6, where the −3σMADe limit fell below 1.0 $\log_{10}$ cfu/g, the minimum score given for results below this level, including results where no *Campylobacter* spp. were reported, was adjusted.

In cases when duplicate vials were used in the PT (sample No. 1 and 3 and No. 5 and 9, respectively), the median and σMADe were calculated both for each single sample and for each pair of samples prepared from the same batch of vials (both calculated values are presented in Table 4). The paired values were used for the final performance evaluation, thus using the same scoring limits for both samples in a specific pair.

An overall assessment of the 10 enumerations was performed by summarising all the scores for each NRL. A five-level grading scale was used for the overall assessment: excellent, good, acceptable, needs improvement and poor. "Excellent performance" was considered if all enumerations were within median values ±2σMADe and no *Campylobacter* spp. were reported in the two samples negative for *Campylobacter*, 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".

Assessment of performance in identification

The performance in correctly identifying the species for the samples where *Campylobacter* was detected, the sensitivity in identification, was categorised on a five-level grading scale. The limits were set at the same levels of sensitivity as the scoring percentages for the enumeration performance grading.

Results

Proficiency test number 31 was distributed to 35 NRLs and 34 of them reported the results of the analysis. Fifteen laboratories started the analysis the same week the samples were dispatched from the EURL, 14 NRLs the week after and five NRLs two weeks after (Table 3).

Enumeration of *Campylobacter* spp. (mandatory)

Of the 34 NRLs that reported results, 27 correctly reported *Campylobacter* spp. in all samples where *Campylobacter* spp. were included and no detection of *Campylobacter* in the samples without *Campylobacter*. One false positive result, of sample No. 10, and eight false negative results, of sample No. 2, 6, 7, and 9, were reported. The median values of the enumerations varied from 1.99 (sample No. 6) to 3.99 (sample No. 7) $\log_{10}$ cfu/g (Figure 1 and Figure 2).

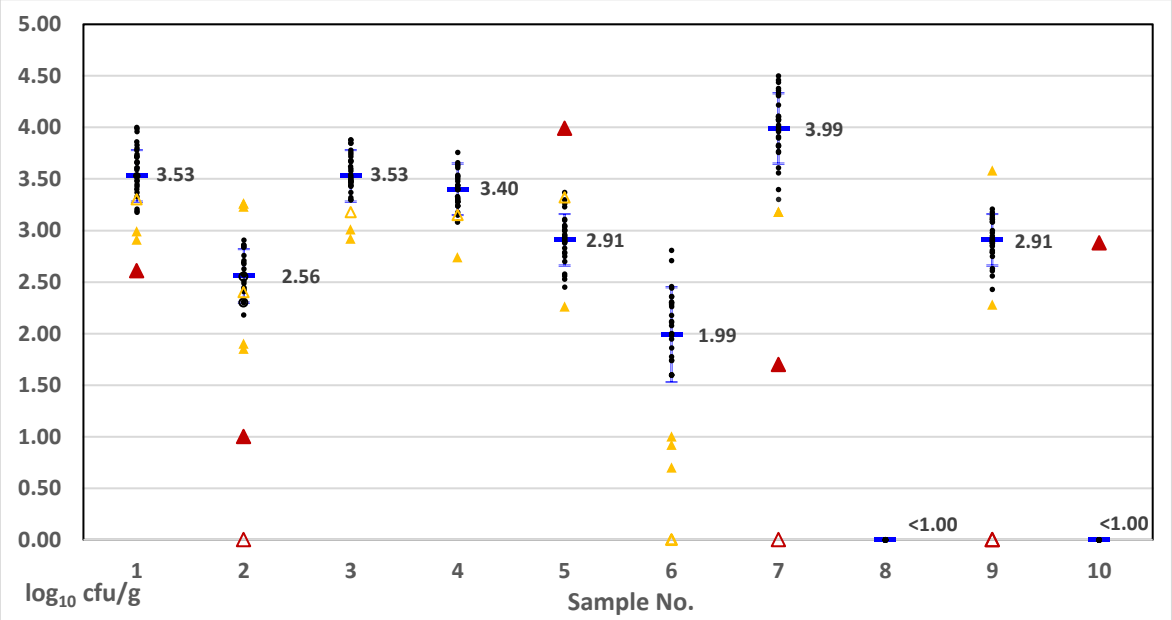

Figure 1. The number ($\log_{10}$ cfu/g) of *Campylobacter* spp. reported by 34 laboratories in PT 31 (2022). The samples reported as *Campylobacter* spp. not detected are shown as 0 in the figure and are represented by non-filled triangles. The median values (for both samples combined in case of duplicate vials) are displayed in numbers and marked with horizontal lines. Vertical bars show the σMADe used in performance evaluation. Results scoring less than the maximum 2 are shown as small (score 1) and large (score 0) triangles, which means that they fall outside the ±2σMADe and ±3σMADe limits, respectively.

Because of low levels detected in sample No. 6, where the −3σMADe limit fell below 1.0 $\log_{10}$ cfu/g, it could not be excluded that negative results (< 1.00 $\log_{10}$ cfu) were proper results that occurred just by chance. Therefore, four negative results were considered as partly acceptable (given the score 1).

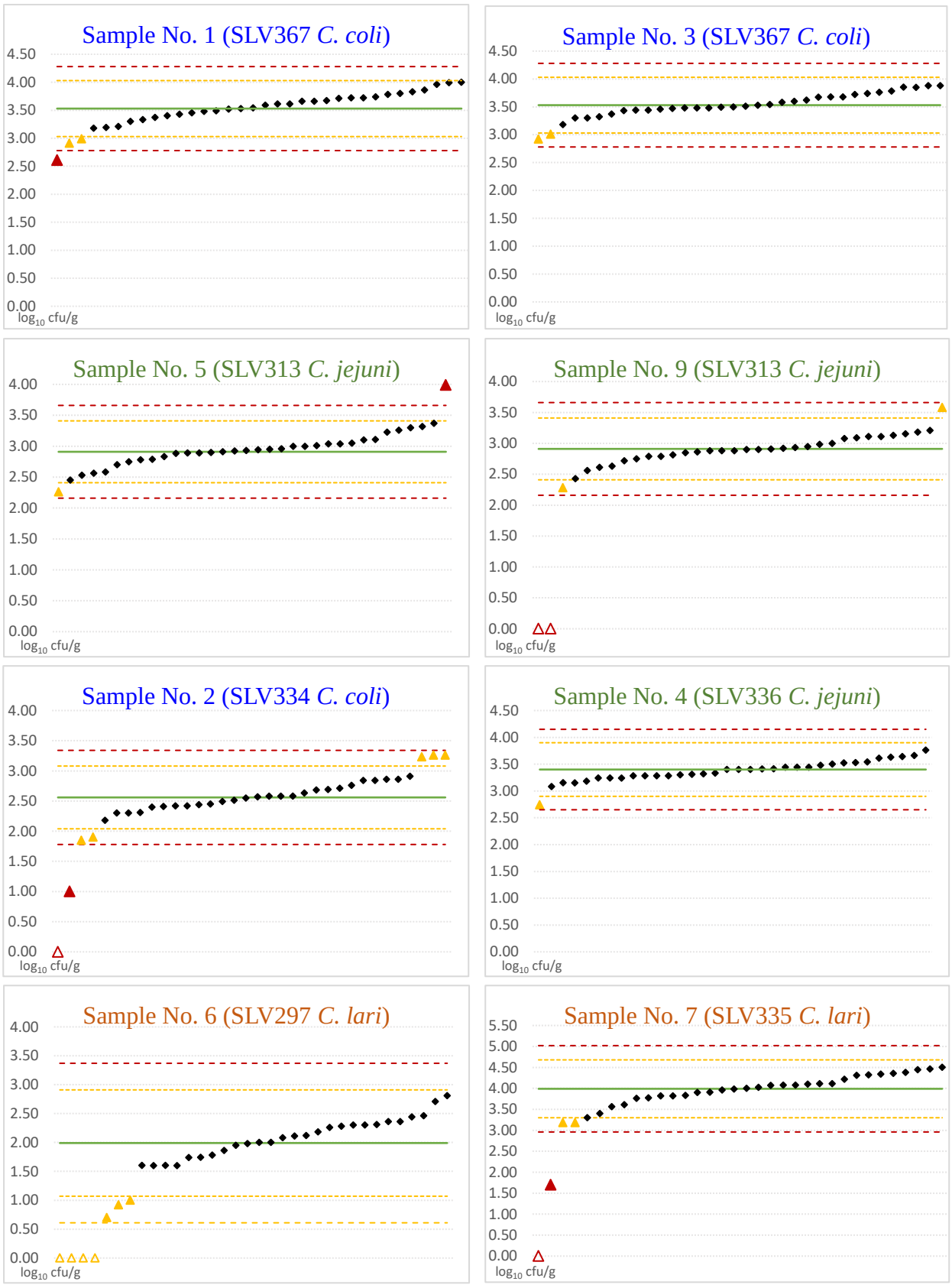

Figure 2. The number (log_{10} cfu/g) of *Campylobacter* spp. reported for each of the eight samples positive for *Campylobacter* by 34 laboratories in PT 31 (2022). Samples reported as *Campylobacter* spp. not detected (< 1.00 log_{10} cfu/g) are shown as 0 in the figure and are represented by non-filled triangles (partly acceptable or unacceptable results) or circles (acceptable results). The median values (for both samples combined in case of duplicate vials) and the ± 2σMADe and ± 3σMADe limits are shown as horizontal lines. Results scoring less than the maximum 2 are shown as small (score 1) and large (score 0) triangles.

Performance in enumeration of *Campylobacter* spp.

The results of using the five-level grading scale for the overall assessment of the NRLs' enumeration of *Campylobacter* spp. are presented in Table 4 and Figure 4.

According to the assessment, 30 NRLs (27 Member State NRLs, MS-NRLs) fulfilled the criterion for excellent or good performance and no MS-NRL scored below the acceptable limit (Table 4 and Figure 3). The overall median percentage of scores was 100 % (50 % Central Range (CR): 85.0 %–100 %).

The NRLs' enumeration results and z-scores for the eight samples positive for *Campylobacter* included in PT 31 are presented in Table 5.

Table 4. Overall performance of the NRLs' enumeration of *Campylobacter* spp. (n=34) in proficiency test No. 31 (2022).

Grade	Scoring limits for each performance grade	Number (proportion) of NRLs with performance within scores	
		All NRLs n=34	MS-NRLs n=28
Excellent	95.1–100%	18 (53%)	18 (64%)
Good	85.0–95.0%	12 (35%)	9 (32%)
Acceptable	70.0–84.9%	4 (12%)	1 (4%)
Needs improvement	57.0–69.9%	0 (0%)	0 (0%)
Poor	< 57.0%	0 (0%)	0 (0%)

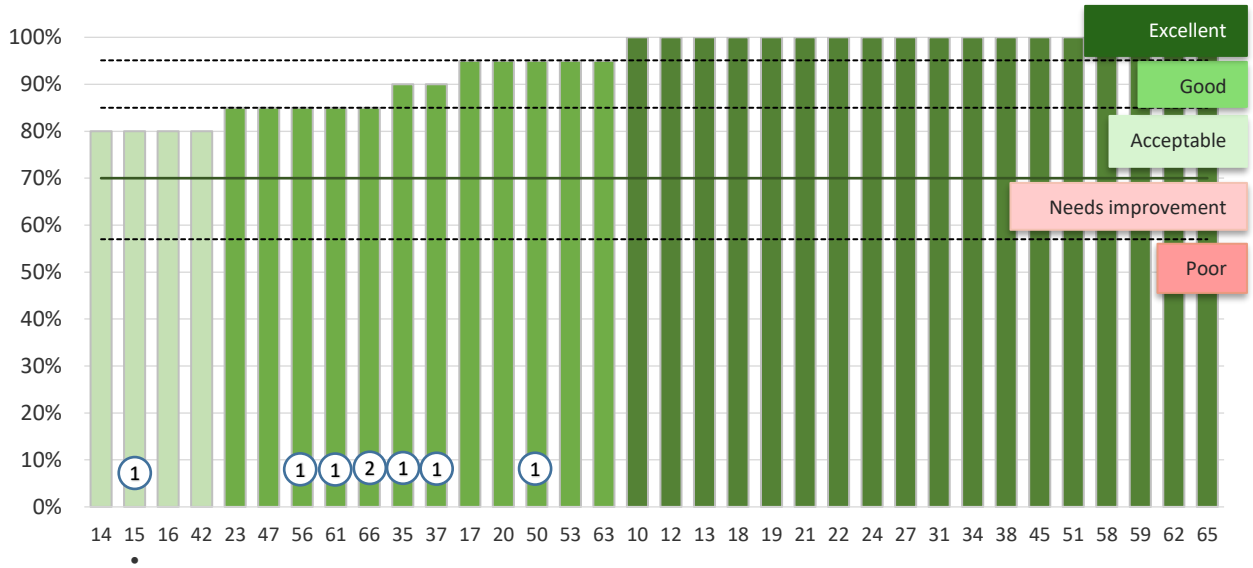

Figure 3. Distribution of the results of participating NRLs (n=34), represented by lab ID, in combined score for enumerations of eight samples with *Campylobacter* and two samples without *Campylobacter* in PT 31 (2022). Limits for grading of the overall performance are marked by horizontal lines. The numbers in white circles denote the number of negative results in samples with *Campylobacter*, and • denotes a false positive result.

Table 5. Results from the enumeration and z-scores of samples with *Campylobacter* in proficiency test No. 31 (2022). Yellow shadowed cells indicate results scoring 1, with median values outside ± 2σMADe and z-scores ± 2.0. Red shadowed cells indicate results scoring 0, with median values outside ± 3σMADe and z-scores ± 3.0. Some scoring adjustments are explained in footnotes.

	Sample 1		**Sample 2**		**Sample 3**		**Sample 4**		**Sample 5**		**Sample 6**		**Sample 7**		**Sample 9**	
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	4.00	1.88	2.63	0.27	3.72	0.76	3.54	0.56	2.94	0.12	1.86	-0.28	4.34	1.03	3.11	0.80
12	3.66	0.52	2.86	1.16	3.76	0.92	3.63	0.92	3.37	1.84	1.95	-0.09	4.50	1.50	2.61	-1.20
13	3.86	1.32	2.86	1.16	3.78	1.00	3.66	1.04	3.10	0.76	2.81	1.78	4.44	1.32	3.15	0.96
14	2.61	-3.68	1.90	-2.54	3.48	-0.20	2.74	-2.64	2.56	-1.40	2.30	0.67	3.98	-0.03	2.56	-1.40
15	3.54	0.04	2.57	0.04	3.49	-0.16	3.30	-0.40	3.00	0.36	2.11	0.26	4.11	0.35	<1.00	-7.64
16	2.91	-2.48	1.00	-6.01	3.01	-2.08	3.50	0.40	2.79	-0.48	1.60	-0.85	3.82	-0.50	2.86	-0.20[a]
17	3.59	0.24	3.26	2.70	3.44	-0.36	3.41	0.04	2.53	-1.52	2.46	1.02	3.77	-0.65	2.63	-1.12
18	3.37	-0.64	2.51	-0.19	3.44	-0.36	3.24	-0.64	3.23	1.28	2.31	0.70	3.56	-1.26	3.13	0.88
19	3.19	-1.36	2.49	-0.27	3.88	1.40	3.31	-0.36	2.93	0.08	2.44	0.98	3.91	-0.23	2.91	0.00
20	3.21	-1.28	2.44	-0.46	3.48	-0.20	3.44	0.16	2.91	0.00	0.70	-2.15	3.76	-0.67	2.92	0.04
21	3.49	-0.16	2.84	1.08	3.58	0.20	3.40	0.00	3.04	0.52	<1.60[b]	-0.85	3.40	-1.73	3.11	0.80
22	3.72	0.76	2.42	-0.54	3.48	-0.20	3.28	-0.48	2.89	-0.08	2.26	0.59	4.08	0.26	2.79	-0.48
23	2.99	-2.16	1.85	-2.74	2.92	-2.44	3.28	-0.48	2.83	-0.32	2.08	0.20	3.61	-1.11	2.95	0.16
24	3.52	-0.04	2.58	0.08	3.43	-0.40	3.28	-0.48	2.78	-0.52	<1.60[b]	-0.85	3.83	-0.47	2.79	-0.48
27	3.74	0.84	2.31	-0.96	3.50	-0.12	3.28	-0.48	3.00	0.36	2.28	0.63	3.96	-0.09	2.88	-0.12
31	3.83	1.20	2.68	0.46	3.74	0.84	3.40	0.00	2.92	0.04	2.30	0.67	4.11	0.35	2.98	0.28
34	3.96	1.72	2.45	-0.42	3.88	1.40	3.53	0.52	3.04	0.52	2.18	0.41	4.32	0.97	3.08	0.68
35	3.99	1.84	<1.00[a]	-6.01	3.53	0.00	3.24	-0.64	3.11	0.80	2.12	0.28	4.07	0.23	3.21	1.20
37	3.40	-0.52	2.30	-1.00	3.32	-0.84	3.18	-0.88	2.70	-0.84	2.00	0.02	4.08	0.26	<1.00[a]	-7.64
38	3.48	-0.20	2.69	0.50	3.60	0.28	3.40	0.00	2.96	0.20	2.00	0.02	4.38	1.14	2.90	-0.04
42	3.67	0.56	2.91	1.35	3.68	0.60	3.33	-0.28	3.99	4.32	1.00	-2.15	3.18	-2.38	2.81	-0.40
45	3.45	-0.32	2.58	0.08	3.37	-0.64	3.44	0.16	2.95	0.16	1.74	-0.54	3.90	-0.26	2.88	-0.12
47	3.33	-0.80	2.58	0.08	3.47	-0.24	3.48	0.32	3.05	0.56	0.92	-2.33	1.70	-6.72	3.09	0.72
50	3.53	0.00	2.55	-0.04	3.62	0.36	3.44	0.16	3.26	1.40	<1.00[a]	-2.15[a]	4.22	0.67	3.00	0.36
51	3.61	0.32	2.84	1.08	3.51	-0.08	3.61	0.84	3.30	1.56	2.36	0.81	4.36	1.09	3.18	1.08
53	3.43	-0.40	2.30	-1.00	3.30	-0.92	3.32	-0.32	2.75	-0.64	<1.60[b]	-0.85[a]	3.18	-2.38	2.72	-0.76
56	3.18	-1.40	2.18	-1.46	3.30	-0.92	3.08	-1.28	2.26	-2.60	<1.00	-2.15	3.30	-2.02[c]	2.28	-2.52
58	3.80	1.08	2.76	0.77	3.85	1.28	3.76	1.44	3.01	0.40	2.71	1.57	4.31	0.94	2.90	-0.04
59	3.72	0.76	2.42	-0.54	3.46	-0.28	3.24	-0.64	2.90	-0.04	1.74	-0.54[a]	4.10	0.32	2.93	0.08
61	3.78	1.00	3.26	2.70	3.54	0.04	3.41	0.04	2.45	-1.84	<1.00	-2.15	3.82	-0.50	3.58	2.68
62	3.71	0.72	2.71	0.58	3.85	1.28	3.64	0.96	2.89	-0.08	1.98	-0.02	4.46	1.38	2.85	-0.24
63	3.66	0.52	3.23	2.58	3.68	0.60	3.52	0.48	2.58	-1.32	2.36	0.81	4.00	0.03	2.43	-1.92
65	3.61	0.32	2.41	-0.58	3.67	0.56	3.15	-1.00	2.88	-0.12	1.78	-0.46[a]	4.02	0.09	2.88	-0.12
66	3.30	-0.92	2.40	-0.62	3.18	-1.40	3.15	-1.00	3.32	1.64	<1.00	-2.15	<1.00[a]	-8.77[a]	2.75	-0.64
Median[d]	**3.53**	3.57	2.56		**3.53**	3.51	3.40		**2.91**	2.94	1.99		3.99		**2.91**	2.89
MADe	**0.16**	0.16	0.18		**0.16**	0.14	0.12		**0.13**	0.12	0.31		0.23		**0.13**	0.11
σMADe	**0.25**	0.25	0.26		**0.25**	0.25	0.25		**0.25**	0.25	0.46		0.34		**0.25**	0.25
±2σMADe	4.03	3.03	3.08	2.04	4.03	3.03	3.90	2.90	3.41	2.41	2.91	1.07	4.68	3.30	3.41	2.41
±3σMADe	4.28	2.78	3.34	1.78	4.28	2.78	4.15	2.65	3.66	2.16	3.37	0.61	5.02	2.96	3.66	2.16

[a] Calculated from 1.00 log_{10} cfu/g.

[b] Reported as "present but lower than 1.60 log_{10} cfu/g", calculations and evaluation based on 1.60.

[c] Rounded to −2.0 and considered on the limit, not exceeding it.

[d] Median value of results for both samples of duplicate vials (No. 1 and 3, and 5 and 9, respectively) in bold, used in performance evaluation, and median value of results for the single sample to the right in blue (with the corresponding MADe and σMADe values in the rows below).

Species identification of *Campylobacter* spp. (voluntary)

Thirty (91 %) of the 34 NRLs reported results of species identification. Two mis-identifications were reported each of sample No. 3 and 5 (Table 6). Twenty-four of the 30 NRLs reported correct species in all eight samples that had been inoculated with *Campylobacter* spp., and 27 NRLs correct species in all inoculated samples where *Campylobacter* spp. had been enumerated (Figure 4).

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

Nineteen of the 30 NRLs reported that they used MALDI-TOF MS for the species identification, in six cases in combination with other techniques. Twelve NRLs used one or more PCR assays, in six cases in combination with other techniques. Seven NRLs reported to have used or adapted the multiplex PCR assay published by Wang *et al.* (2002). There were no other protocols reported to be used by more than one NRL. Nine NRLs used biochemical tests (at least detection of catalase), in seven cases in combination with MALDI-TOF MS or PCR.

Twenty NRLs used one technique only (a set of biochemical tests regarded as one technique) and ten NRLs combined two techniques for the species identification.

Table 6. Species identification reported by 30 NRLs in the voluntary part of proficiency test No. 31 (2022).

		Number of NRLs reporting				
Content of sample (vial)		*Campylobacter jejuni*	*Campylobacter coli*	*Campylobacter lari*	No growth at all	Growth of other, not *Campylobacter*
1.	*Campylobacter coli*		30			
2.	*Campylobacter coli*		29			1
3.	*Campylobacter coli*	2	28			
4.	*Campylobacter jejuni*	30				
5.	*Campylobacter jejuni*	28	1	1		
6.	*Campylobacter lari*			28	2	
7.	*Campylobacter lari*	1		29		
8.	*Escherichia coli*				3	27
9.	*Campylobacter jejuni*	28			1	1
10.	Negative	1			29	

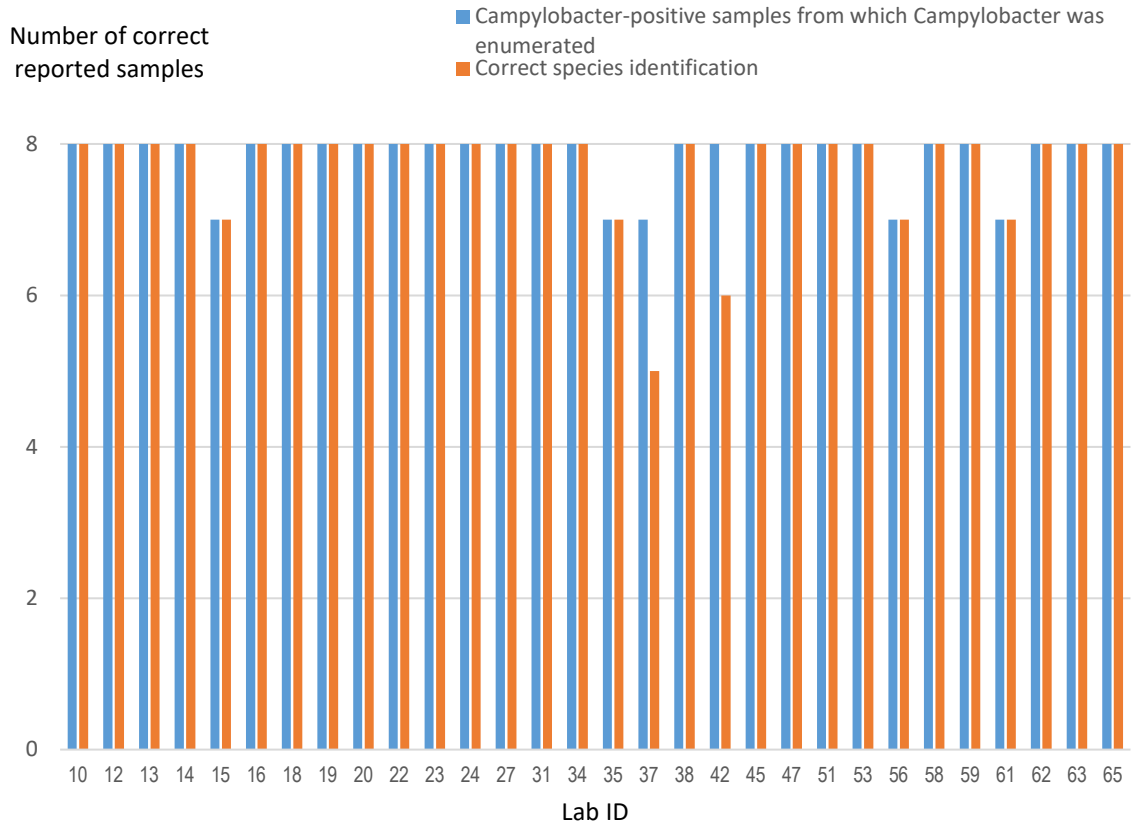

Figure 4. Results by 30 NRLs reporting results for species identification in the voluntary part of proficiency test No. 31 (2022).

Performance in identification of *Campylobacter* spp.

All 30 NRLs reporting results for species identification of *Campylobacter* fulfilled the criterion for at least acceptable performance in identification of *Campylobacter* spp. (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 31 (2022).

		Identification of *Campylobacter* spp.	
Grade	**Sensitivity**	**Number of NRLs (%) All NRLs, n=30**	**Number of NRLs (%) MS-NRLs, n=25**
Excellent	95.1–100%	28 (93%)	24 (96%)
Good	85.0–95.0%	0 (0%)	0 (4%)
Acceptable	70.0–84.9%	2 (7%)	1 (4%)
Needs improvement	57.0–69.9%	0 (0%)	0 (0%)
Poor	<57.0%	0 (0%)	0 (0%)

References

ISO 10272-2:2017: Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of *Campylobacter* spp. – Part 2: Colony-count technique. International Organization for Standardization.

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Wang GH, Clark CG, Taylor TM, Pucknell C, Barton C, Price L, Woodward, DL, Rodgers, FG. Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus* subsp. *fetus*. Journal of Clinical Microbiology. 2002;40(12):4744–7. doi: 10.1128/JCM.40.12.4744-4747.2002