EURL-*CAMPYLOBACTER*

REPORT

PROFICIENCY TEST NUMBER 34

Enumeration (and voluntary species identification) of

Campylobacter

Publication history

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Contents

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
No.	number
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 34, 2023

The EU reference laboratory for *Campylobacter* organised proficiency test (PT) number 34 on enumeration of *Campylobacter* spp. in chicken skin in March 2023. The PT included enumeration of *Campylobacter* spp. in ten 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 a voluntary part of PT 34.

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

Thirty (86 %) NRLs fulfilled the criterion for excellent or good performance in enumeration of *Campylobacter* spp., and three NRLs (two Member State NRLs, MS-NRLs) scored below the acceptable limit. Thirty-one of the 35 NRLs reported results of species identification of *Campylobacter*, and 30 (97 %) of them fulfilled the criterion for excellent performance in identification of *Campylobacter* spp. One NRL (no MS-NRL) scored below the acceptable limit. Only one misidentification was reported.

In summary, the majority of the NRLs met the criteria for excellent or good performance in both enumeration and species identification, and three NRLs scored below the acceptable limit in enumeration. The underperforming MS-NRLs were offered and performed an extra PT.

Introduction

Proficiency test (PT) number 34 on enumeration of *Campylobacter* spp. in chicken skin was organised by the EU reference laboratory (EURL) for *Campylobacter* in March 2023. Thirty-five national reference laboratories (NRLs) in 27 EU Member States (some Member States have more than one NRL) and in Iceland, Norway, Republic of North Macedonia, and United Kingdom received the PT. All 35 NRLs reported the test results and operational details to the EURL.

Thirty-four NRLs reported that they were accredited for detection of *Campylobacter* and 28 that they were accredited for enumeration of *Campylobacter*. Six NRLs were accredited for detection only, and one NRL was accredited neither for detection nor enumeration of *Campylobacter* spp.

The PT included enumeration of *Campylobacter* spp. in ten 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 a voluntary part of PT 34.

Table 1. Contents of the ten vials distributed to the NRLs in proficiency test No. 34, 2023.

Sample No.	Species	Level [b] (log_{10} cfu/vial)	Standard deviation [b] (log_{10} cfu)	Batch No.
1	*Campylobacter jejuni* [a]	4.19	0.07	SLV313
	+ *Escherichia coli*	3.55	0.07	
2	*Campylobacter lari*	4.86	0.08	SLV335
3	*Campylobacter coli*	6.67	0.06	SLV374
4	*Campylobacter coli*	5.36	0.15	SLV333
5	Negative			
6	*Campylobacter lari*	4.86	0.08	SLV335
7	*Campylobacter coli*	6.67	0.06	SLV374
8	*Escherichia coli*	4.29	0.06	SVA079
9	*Campylobacter jejuni* [a]	3.81	0.09	SLV306
10	*Campylobacter jejuni* [a]	4.19	0.07	SLV313
	+ *Escherichia coli*	3.55	0.07	

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

[b] According to homogeneity test of ten 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 one year. The broilers were slaughtered at a slaughterhouse with a history of low level of *Campylobacter*-positive flocks (3.1 % during 2022).

The chicken thigh skin was tested on arrival in triplicate with enrichment in Bolton and Preston broth and by direct streak from each initial suspension on modified charcoal cefoperazone deoxycholate (mCCD) agar and Preston agar. The chicken skin tested negative for presence of *Campylobacter* but a moderate background flora was present. In addition, caecal samples from the same chicken flock tested negative for *Campylobacter*. The chicken skin was cut in smaller pieces, divided 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 the EURL and tested for stability and homogeneity by the producer. The standard deviation from the homogeneity testing of ten vials analysed in repeatable conditions is included in Table 1. Before choosing the vials for the PT, the EURL tested at least two vials (three when the batch was not previously tested by the EURL) of each batch on 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 several occasions (Table 2). These tests were performed before dispatch on vials stored in “best case” transport conditions (5 °C for 24 h). They were also performed two days after dispatch (“best case” conditions) and two weeks after dispatch, at the last date for start of analysis by the participants, on vials stored in “worst case” conditions (5 °C for 24 h, 15 °C for 24 h, and 5 °C for 24 h) before storage at −20 °C until start of analysis.

The levels of *Campylobacter* in vials stored in "worst case" conditions were similar (both higher and lower) to those stored in "best case" conditions. The variability of all tests under variable technical (different time points, personnel, equipment, and media batch) and transport conditions (both "best case" and "worst case") was evaluated per used vial (6 or 7 of each according to Table 2). The variation observed (with a range of 0.42 $\log_{10}$ cfu for SLV313 to 1.23 $\log_{10}$ cfu for SLV306) was accounted the variability of each vial and technical variation of the method. The method for assessment of performance, which took the actual results and variability between participants into account, was deemed adequate, with no further adjustments needed.

Table 2. Outline of stability testing under transport conditions for proficiency test No. 34, 2023.

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

[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 20th of March, 2023. The samples were placed in styrofoam boxes along with freezing blocks. The styrofoam boxes were packed in cardboard boxes for transport and were sent from the EURL with 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 about 120 g of frozen chicken skin. The skin was to be divided into 10 g portions, one for each of the ten vials. A Micro-T-Log was included in each package to record the temperature every second hour during transport.

Thirty NRLs received the PT within one day after the packages had been dispatched from the EURL, four NRLs within two days, and one NRL after three days (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 3rd of April. 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. 34, 2023.

Arrival	**Number of NRLs n=35**	**Start of analysis**	**Number of NRLs n=35**
21st of March	30	21st of March	2
22nd of March	4	22nd of March	11
23rd of March	1	23rd of March	3
		24th of March	4
		27th of March	8
		28th of March	3
		29th of March	2
		31st of March	1
		12th of April	1

Methods for analysis

The NRLs were recommended to follow ISO 10272-2:2017 for performing PT 34. However, if their standard laboratory procedure followed a different method, they were allowed to use that method for the test.

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 35 NRLs, 23 reported using commercial gas-generating kits, eight microaerobic incubators, seven the Anoxomat® system and one another method (GENbox Microaer gas generator). 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 two samples with the most homogeneous results (sample No. 2 and 6), 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.

In cases when duplicate vials were used in the PT (sample No. 1 and 10, No. 2 and 6, and No. 3 and 7, 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 ten 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" was 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 34 was distributed to 35 NRLs and all of them reported the results of the analysis.

According to the instructions, analysis of the samples should be started the same week as the samples were dispatched from the EURL, and no later than two weeks after dispatch. Twenty laboratories started the analysis the same week the samples were dispatched from the EURL, 14 NRLs the week after and one NRLs three weeks after (Table 3).

Thirty-three NRLs reported to have followed the recommended method ISO 10272-2:2017, either the originally publicated method (18), including ISO 10272-2:2017/Amd 1:2023 (14), or a combination of the two (1). Two NRLs used other methods: NMKL 119 3rd ed., 2007, and an internal method, respectively.

Enumeration of *Campylobacter* spp. (mandatory)

Of the 35 NRLs, 33 correctly reported *Campylobacter* spp. in all samples containing *Campylobacter* spp. and no detection of *Campylobacter* in the samples without *Campylobacter*. Two false negative results, of sample No. 2 and 9, were reported. The median values of the enumerations varied from 2.64 (sample No. 9) to 5.64 (sample No. 3 and 7) $\log_{10}$ cfu/g (Figure 1 and Figure 2).

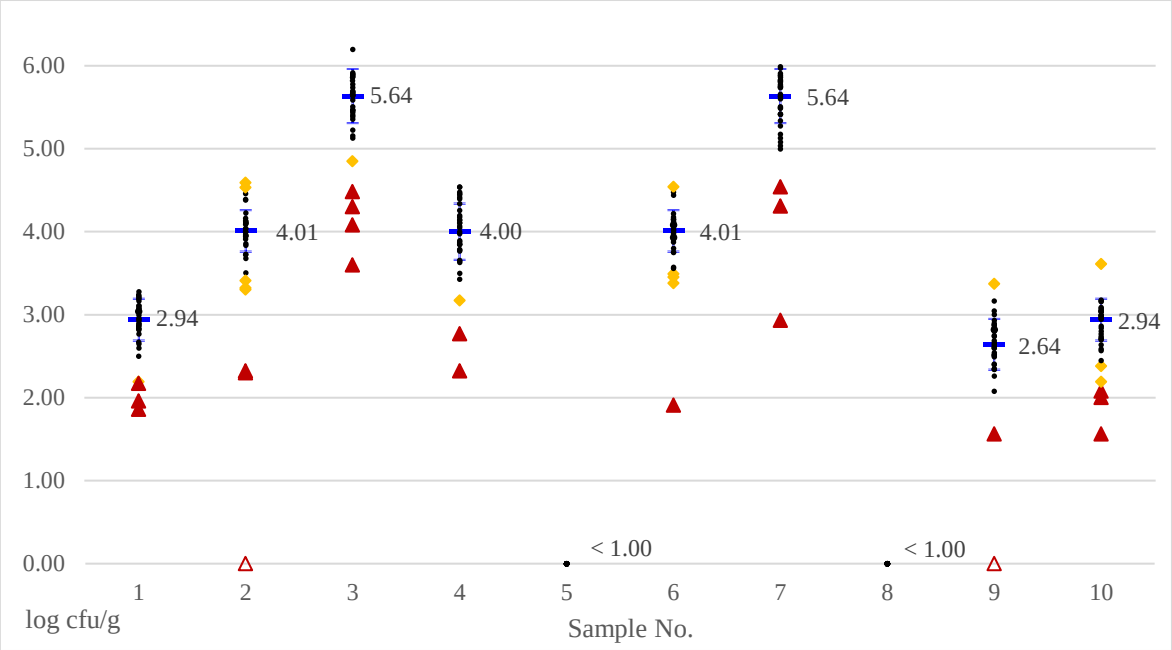

Figure 1. The quantity ($\log_{10}$ cfu/g) of *Campylobacter* spp. reported by 35 laboratories in proficiency test No. 34, 2023. The samples reported as *Campylobacter* spp. not detected are shown as 0 in the figure and false negatives are represented by non-filled triangles. The median values (for both 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 filled diamonds (score 1) or triangles (score 0), which means that they fall outside the ± 2σMADe and ± 3σMADe limits, respectively.

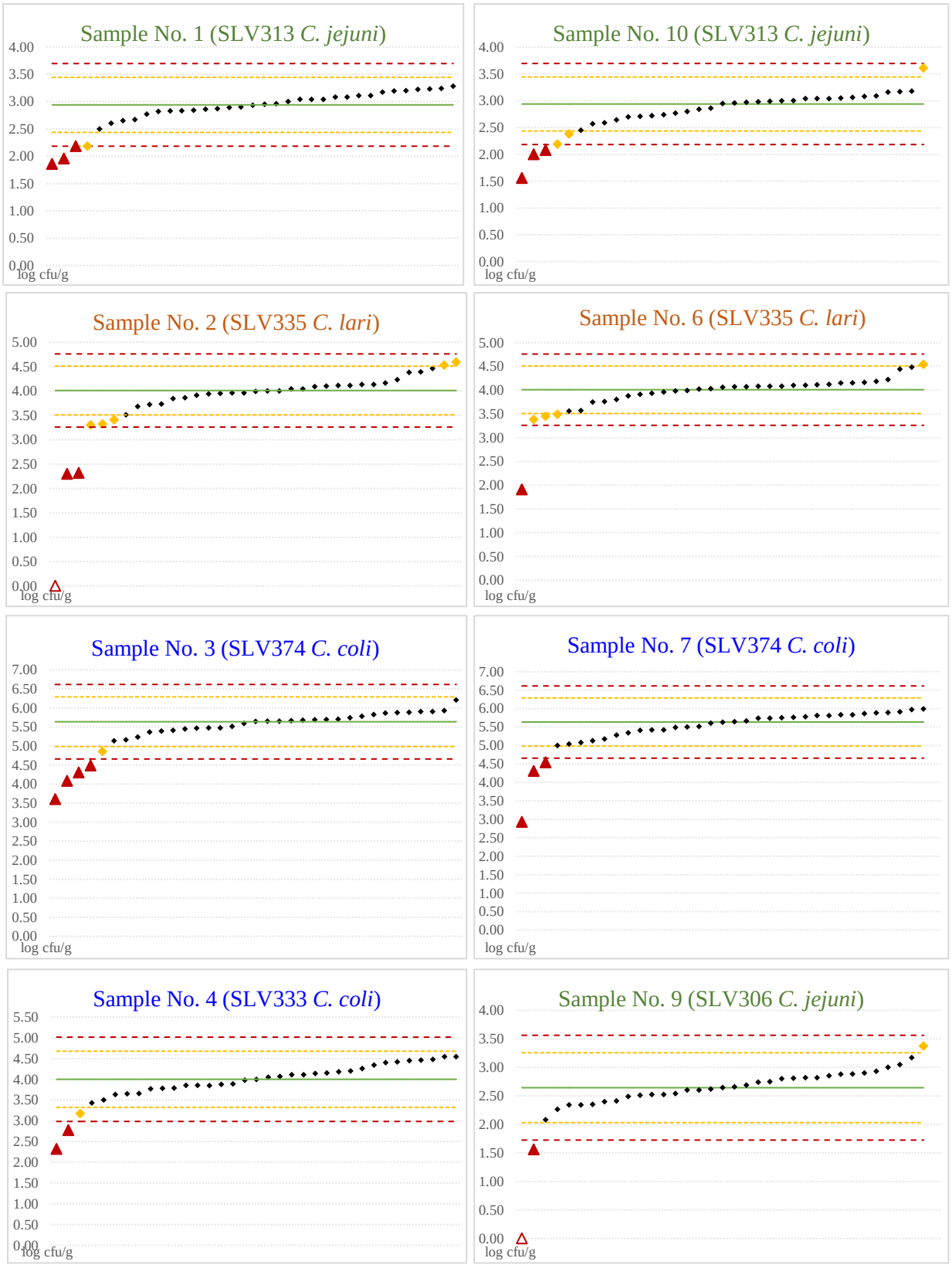

Figure 2. The quantity (log_{10} cfu/g) of *Campylobacter* spp. reported for each of the eight samples positive for *Campylobacter* by 35 NRLs in proficiency test No. 34, 2023. Samples reported as *Campylobacter* spp. not detected (< 1.00 log_{10} cfu/g) are shown as 0 in the figure and are represented by non-filled triangles. 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 diamonds (score 1) or triangles (score 0).

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 3.

According to the assessment, 30 NRLs (26 Member State NRLs, MS-NRLs) fulfilled the criterion for excellent or good performance and three NRLs (two MS-NRLs) scored below the acceptable limit (Table 4 and Figure 3). The overall median percentage of scores was 100 % (50 % Central Range (CR): 90.0 %–100 %).

The NRLs' enumeration results and z-scores for the eight samples positive for *Campylobacter* included in PT 34 are presented in Table 5.

Table 4. Overall performance of 35 NRLs' enumeration of *Campylobacter* spp. in proficiency test No. 34, 2023.

Grade	Scoring limits for each performance grade	Number (proportion) of NRLs with performance within scores	
		All NRLs n=35	MS-NRLs n=29
Excellent	95.1–100 %	20 (57 %)	18 (62 %)
Good	85.0–95.0 %	10 (29 %)	8 (28 %)
Acceptable	70.0–84.9 %	2 (6 %)	1 (3 %)
Needs improvement	57.0–69.9 %	1 (3 %)	0 (0 %)
Poor	< 57.0 %	2 (6 %)	2 (7 %)

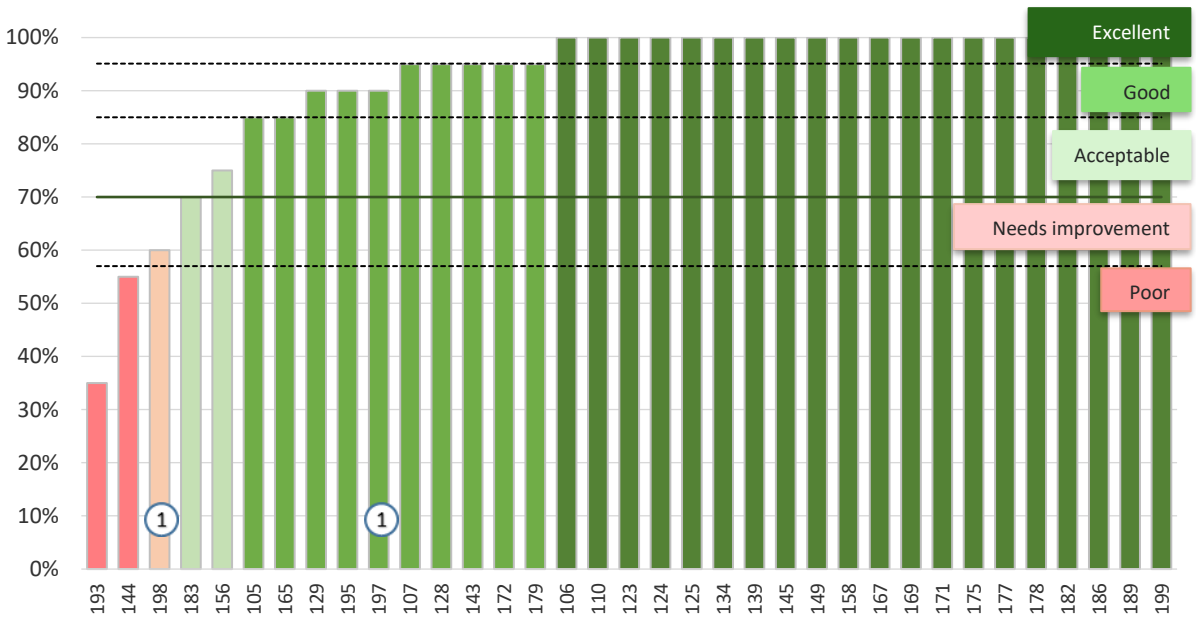

Figure 3. Distribution of the results of participating NRLs (n=35), represented by lab ID, in combined score for enumerations of eight samples with *Campylobacter* and two samples without *Campylobacter* in proficiency test No. 34, 2023. Limits for grading of the overall performance are marked by horizontal lines. The numbers in white circles denote the number of negative results in samples containing *Campylobacter*.

Table 5. Results from the enumeration and z-scores of samples with *Campylobacter* in proficiency test No. 34, 2023. 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 6**		**Sample 7**		**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
105	3.04	0.40	3.32	-2.76	5.74	0.32	3.85	-0.44	3.45	-2.24	5.83	0.60	3.37	2.37	3.00	0.24
106	2.95	0.04	3.73	-1.12	5.59	-0.14	4.18	0.53	4.15	0.56	5.81	0.54	2.75	0.35	2.98	0.16
107	3.23	1.15	4.59	2.32	5.39	-0.75	3.85	-0.44	4.48	1.88	5.91	0.84	3.05	1.33	3.08	0.56
110	2.83	-0.44	3.99	-0.08	5.69	0.17	3.89	-0.32	4.11	0.40	5.66	0.08	2.52	-0.40	2.59	-1.39
123	3.20	1.03	4.00	-0.04	5.51	-0.38	4.40	1.18	4.18	0.68	5.81	0.54	2.82	0.58	3.04	0.40
124	3.19	0.99	4.13	0.48	5.86	0.69	4.14	0.41	4.12	0.44	5.78	0.44	2.88	0.77	3.00	0.24
125	3.24	1.19	4.39	1.52	5.78	0.44	4.45	1.33	4.22	0.84	5.34	-0.90	2.82	0.58	3.18	0.95
128	2.82	-0.48	3.96	-0.20	4.85	-2.41	3.79	-0.62	4.07	0.24	5.00	-1.95	2.35	-0.96	2.72	-0.87
129	2.19	-2.98	3.86	-0.60	5.65	0.05	4.07	0.21	4.07	0.24	5.50	-0.41	2.34	-0.99	2.38	-2.22
134	2.90	-0.16	4.13	0.48	5.36	-0.84	4.42	1.24	4.08	0.28	5.42	-0.66	2.69	0.15	3.05	0.44
139	2.87	-0.28	3.91	-0.40	5.41	-0.69	3.65	-1.03	3.91	-0.40	5.42	-0.66	2.26	-1.25	2.64	-1.19
143	2.65	-1.15	3.72	-1.16	5.47	-0.51	3.17	-2.45	4.02	0.04	5.04	-1.82	2.41	-0.76	2.57	-1.47
144	2.67	-1.07	3.30	-2.84	3.60	-6.24	3.43	-1.68	3.76	-1.00	2.93	-8.29	1.56	-3.54	1.56	-5.48
145	3.08	0.56	4.11	0.40	5.68	0.14	3.66	-1.00	3.88	-0.52	5.28	-1.09	2.85	0.67	2.99	0.20
149	3.22	1.11	4.16	0.60	5.92	0.87	4.54	1.59	4.10	0.36	5.89	0.78	2.90	0.84	3.17	0.91
156	1.96	-3.89	3.51	-2.00[b]	4.30	-4.09	3.77	-0.67	3.49	-2.08	5.18	-1.41	2.64	0.00	2.96	0.06
158	2.60	-1.35	4.23	0.88	5.64	0.02	4.00	0.00	4.15	0.56	5.88	0.75	2.34	-0.99	3.04	0.40
165	2.93	-0.04	4.46	1.80	6.20	1.73	4.54	1.59	4.54	2.12	5.99	1.09	2.08	-1.84	2.00	-3.73
167	2.83	-0.44	4.09	0.32	5.70	0.20	3.98	-0.06	3.99	-0.08	5.63	-0.02	2.74	0.32	3.09	0.60
169	2.50	-1.75	3.96	-0.20	5.65	0.05	3.85	-0.44	3.98	-0.12	5.51	-0.38	2.62	-0.08	2.45	-1.94
171	2.77	-0.67	3.68	-1.32	5.16	-1.46	3.50	-1.48	3.75	-1.04	5.41	-0.69	2.60	-0.14	2.74	-0.79
172	2.96	0.08	4.10	0.36	5.13	-1.55	4.34	1.00	4.16	0.60	5.49	-0.44	2.66	0.05	3.61	2.66
175	3.28	1.35	4.38	1.48	5.44	-0.60	3.78	-0.65	4.44	1.72	5.60	-0.11	3.17	1.72	3.16	0.87
177	3.04	0.40	3.94	-0.28	5.82	0.57	4.11	0.32	3.93	-0.32	5.74	0.32	2.93	0.94	2.77	-0.67
178	3.00	0.24	3.95	-0.24	5.46	-0.54	4.15	0.44	4.08	0.28	5.76	0.38	2.88	0.77	2.97	0.12
179	3.17	0.91	4.53	2.08	5.88	0.75	4.20	0.59	4.10	0.36	5.86	0.69	2.81	0.54	3.06	0.48
182	3.08	0.56	4.04	0.12	5.90	0.81	4.46	1.36	4.08	0.28	5.97	1.03	2.80	0.51	2.86	-0.32
183	3.11	0.67	3.41	-2.40	5.67	0.11	3.88	-0.35	3.38	-2.52	4.54	-3.36	2.60	-0.14	2.08	-3.41
186	2.86	-0.32	3.84	-0.68	5.47	-0.51	4.05	0.15	3.80	-0.84	5.64	0.02	2.49	-0.50	2.80	-0.56
189	2.84	-0.40	4.00	-0.04	5.66	0.08	3.63	-1.09	4.06	0.20	5.74	0.32	2.52	-0.40	2.71	-0.91
193	1.86	-4.29	2.30	-6.84	4.08	-4.77	2.32	-4.96	1.91	-8.40	4.31	-4.06	2.51	-0.44	2.19	-2.98
195	2.89	-0.20	2.32	-6.76	5.87	0.72	4.11	0.32	3.56	-1.80	5.83	0.60	2.40	-0.80[a]	2.70	-0.95
197	3.04	0.40	4.04	0.12	5.23	-1.24	4.48	1.42	4.03	0.08	5.13	-1.55	<1.00	-5.37	2.95	0.04
198	2.17	-3.06	<1.00	-12.04[a]	4.48	-3.54	2.77	-3.63	3.57	-1.76	5.08	-1.70	2.54	-0.34	2.84	-0.40
199	3.11	0.67	4.11	0.40	5.90	0.81	4.26	0.77	3.96	-0.20	5.75	0.35	3.00	0.40	3.04	1.16
Median[c]	**2.94**	2.93	**4.01**	3.99	**5.64**	5.64	4.00		**4.01**	4.06	**5.64**	5.63	2.64		**2.94**	2.95
MADe	**0.17**	0.16	**0.14**	0.15	**0.22**	0.22	0.23		**0.14**	0.10	**0.22**	0.21	0.21		**0.17**	0.18
σMADe	**0.25**	0.25[d]	**0.25**[d]	0.25[d]	**0.33**	0.33	0.34		**0.25**[d]	0.25[d]	**0.33**	0.31	0.31		**0.25**	0.27
±2σMADe	3.45	2.43	4.51	3.51	6.29	4.98	4.68	3.32	4.51	3.51	6.29	4.98	3.26	2.03	3.45	2.43
±3σMADe	3.70	2.18	4.76	3.26	6.62	4.65	5.02	2.98	4.76	3.26	6.62	4.65	3.57	1.72	3.70	2.18

[a] z-score calculated from 1.00 log_{10} cfu/g.

[b] z- score considered to be on the limit −2.0, not exceeding it.

[c] Median value of results for both samples of duplicate vials (No. 1 and 10, 2 and 6, and 3 and 7, 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).

[d] Adjusted according to the 0.5 log_{10} rule (ISO 22117:2019).

Species identification of *Campylobacter* spp. (voluntary)

Thirty-one (89 %) of the 35 NRLs reported results of species identification. One NRL could not specify any of the three *C. jejuni* samples, but erroneously reported sample No. 9 as *C. coli*, and could not identify species of sample No. 1 and 10 (Table 6). Twenty-nine of the 31 NRLs reported correct species in all eight samples that had been inoculated with *Campylobacter* spp., and 28 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, polymerase chain reaction (PCR) or matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS). The biochemical tests included detection of catalase, hippurate hydrolysis, indoxyl acetate hydrolysis, sensitivity to cephalotin, and hydrogen sulphide production in triple sugar iron medium.

Twenty of the 31 NRLs reported that they used MALDI-TOF MS for the species identification, in seven cases combined with other techniques. Twelve NRLs used one or more PCR assays, in six cases combined with other techniques. Six NRLs reported to have used the multiplex PCR assay published by Wang et al. (2002), and two to have used the multiplex PCR protocol recommended by EURL-AR (2013). Nine NRLs used biochemical tests (at least detection of catalase), in seven cases combined with MALDI-TOF MS or PCR.

Twenty-one 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 31 NRLs in the voluntary part of proficiency test No. 34, 2023.

		Number of NRLs reporting					
Content of sample (vial)		*Campylobacter jejuni*	*Campylobacter coli*	*Campylobacter lari*	*Campylobacter* spp. but unable to identify species	No growth at all	Growth of other, not *Campylobacter*
1.	*C. jejuni* & *E. coli*	30			1		
2.	*C. lari*			31			1
3.	*C. coli*		31				
4.	*C. coli*		31				
5.	Negative					26	5
6.	*C. lari*			31			
7.	*C. coli*		31				
8.	*E. coli*					8	23
9.	*C. jejuni*	30	1				
10.	*C. jejuni* & *E. coli*	30			1		

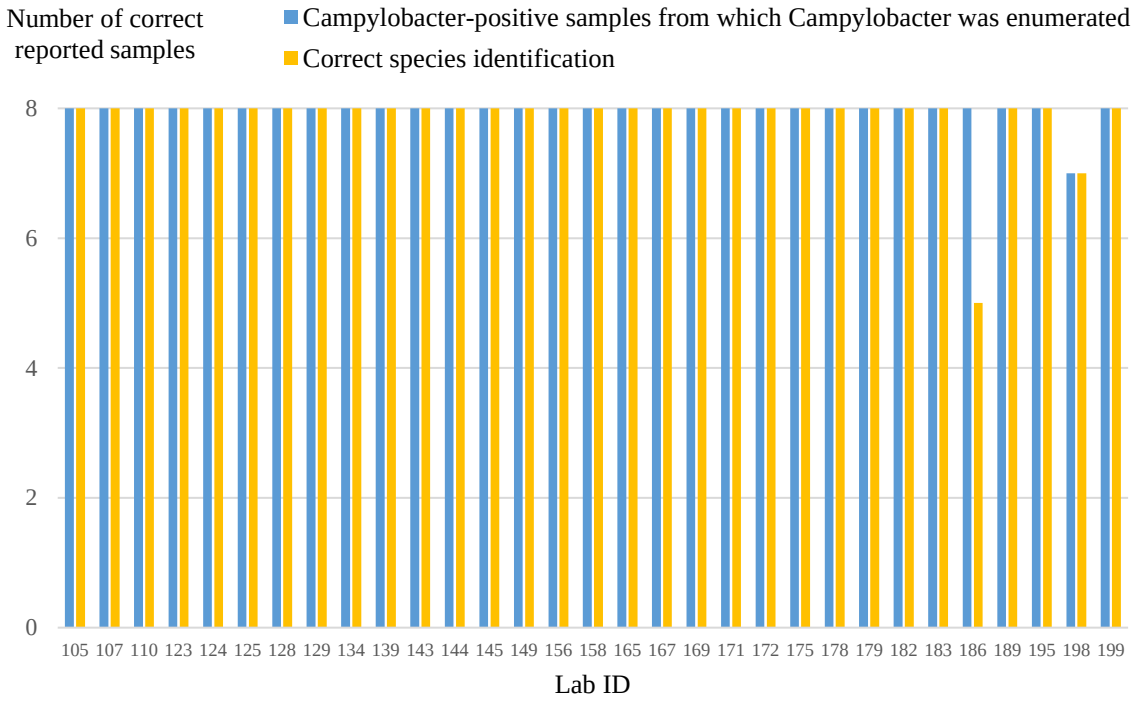

Figure 4. Results by 31 NRLs reporting results for species identification in the voluntary part of proficiency test No. 34, 2023.

Performance in identification of *Campylobacter* spp.

Thirty of the 31 NRLs reporting results for species identification of *Campylobacter* fulfilled the criterion for excellent performance in identification of *Campylobacter* spp., and one NRL (no MS-NRL) scored below the acceptable limit (Table 7). The overall median sensitivity in correctly identifying *Campylobacter* spp. was 100 % (50 % CR: 100 %–100 %).

Table 7. Overall performance of 31 NRLs' sensitivity in correctly identifying *Campylobacter* spp. in the voluntary part of proficiency test No. 34, 2023.

		Performance in identification of *Campylobacter* spp.	
Grade	**Sensitivity**	**Number of NRLs (%) All NRLs, n=31**	**Number of NRLs (%) MS-NRLs, n=25**
Excellent	95.1–100 %	30 (97)	25 (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 %	1 (3)	0 (0)
Poor	< 57.0 %	0 (0)	0 (0)

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