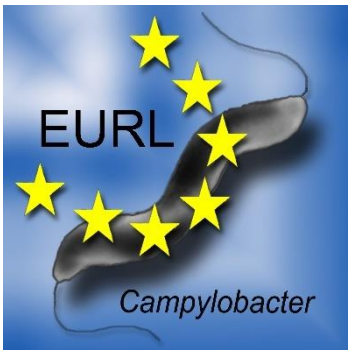

EURL-*CAMPYLOBACTER*

REPORT

PROFICIENCY TEST NUMBER 29

Enumeration (and voluntary species identification) of *Campylobacter*

Publication history

Version	Date
Final version	2021-12-31

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
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
spp.	species

Summary of the proficiency test number 29, 2021

The EU reference laboratory for *Campylobacter* organised proficiency test (PT) number 29 on enumeration of *Campylobacter* spp. in fresh cabbage in March 2021. The PT was designed to enable using parts of the results to validate an additional food category ("fresh produce and fruits") to turn the scope of ISO 10272-2 into "broad-range of foods". The PT included enumeration of *Campylobacter* spp. in 10 samples of shredded cabbage 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 shredded cabbage. Species identification of detected *Campylobacter* was included as a voluntary part of PT 29.

Thirty-three NRLs in 27 EU member states (some member states have more than one NRL) and in Iceland, Norway, and United Kingdom participated in the PT. Thirty-one NRLs reported to have followed the recommended method of ISO 10272-2:2017, and two NRLs used other methods.

Generally, the median results reported for the *Campylobacter*-containing samples were lower than expected, and there was low variability in level between samples. For some samples it could not be excluded that negative results (<1.0 log cfu) were proper results that occurred just by chance. Consequently, some negative results were considered as fully or partly acceptable, according to the used scaled median absolute deviation method for evaluation. The low variability in level between samples intended for the validation study precluded the data from being usable for validation of the method, but could still be used for evaluating the NRLs' performance.

Thirty-one (94%) NRLs fulfilled the criterion for excellent or good performance in enumeration of *Campylobacter* spp., which is a somewhat higher proportion than the four previous years. At the same time, the median percentage of scores was somewhat lower than previous years, since more NRLs got the performance grade *Good* rather than *Excellent*. Two NRLs scored below the acceptable limit.

Twenty-nine (88%) of the 33 NRLs reported results of species identification of *Campylobacter*, and all of them fulfilled the criterion for excellent or good performance in identification of *Campylobacter* spp. Only one misidentification of species was reported.

In summary, the majority of the NRLs met the criteria for excellent or good performance in both enumeration and species identification, and only one Member State NRL scored below the acceptable limit in enumeration. The underperforming NRL has been offered and performed an extra PT.

Introduction

Proficiency test (PT) number 29 on enumeration of *Campylobacter* spp. in fresh shredded cabbage was organised by the EU reference laboratory (EURL) for *Campylobacter* in March 2021. Thirty-three national reference laboratories (NRLs) in 27 EU member states (some member states have more than one NRL) and in Iceland, Norway, and United Kingdom participated in the PT. The test results and operational details were reported to the EURL from all 33 NRLs.

Thirty-one NRLs reported that they were accredited for detection of *Campylobacter* and 28 that they were accredited for enumeration of *Campylobacter*. Four NRLs were accredited for detection only, and one NRL was accredited for enumeration only, while 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 shredded cabbage 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 shredded cabbage. Species identification of detected *Campylobacter* was included as a voluntary part of PT 29.

Table 1. Contents of the 10 vials distributed to the NRLs in proficiency test No. 29 (2021).

Sample No.	Species	Level [b] (log_{10} cfu/vial)	Standard deviation [b] (log_{10} cfu)	Batch No.
1	*Campylobacter lari*	5.22	0.14	SVA049
2	*Campylobacter lari*	4.22	0.10	SVA048
3	*Campylobacter lari*	6.05	0.04	SVA058
4	*Campylobacter lari*	6.05	0.04	SVA058
5	*Campylobacter lari*	4.22	0.10	SVA048
6	*Campylobacter coli*	4.45	0.09	SVA060
7	Negative			
8	*Campylobacter lari*	5.22	0.14	SVA049
9	*Campylobacter jejuni* [a]	4.53	0.09	SVA059
10	*Escherichia coli*	4.74	0.08	SVA045

[a] The strain was hippurate positive.

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

The PT was designed to enable using parts of the results to validate an additional food category to turn the scope of ISO 10272-2 into "broad-range of foods". Therefore, the same strain of *Campylobacter lari* was applied in several samples, in duplicates at three different levels. It was voluntary to participate in the validation study, and the NRLs willing to do so registered interest for this when they registered for the PT. For NRLs participating in the validation study, it was mandatory to follow ISO 10272-2 in detail when performing the test.

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*.
- 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 cabbage

The cabbage used as matrix was bought in a retail shop 15 days before distribution of the PT. The material tested negative for presence of *Campylobacter* but contained a background flora of naturally contaminating bacteria. Several bacterial genera and species were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS): e.g. *Pseudomonas fluorescens*, *Micrococcus luteus*, *Erwinia persicina*, and *Staphylococcus hominis*.

The cabbage was shredded, divided into portions of about 60 g each, and vacuum-packed in plastic bags 14 days before dispatch of the PT. Thereafter, the vacuum-packed cabbage was stored at −4 °C 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 EURL. Before choosing the vials for the PT, the EURL tested three vials of each batch with modified charcoal cefoperazone deoxycholate (mCCD) agar. The results were noted as common logarithm values ($\log_{10}$) of cfu for analysis of each tested vial and values for the difference between the highest and lowest values. The vials chosen for the PT included vials with various *Campylobacter* levels, and the maximum difference allowed between the three tested vials in each batch was 0.50 $\log_{10}$ cfu.

Enumeration of *Campylobacter* spp. in shredded cabbage (of the batch prepared for the PT) according to ISO 10272-2:2017 was performed by the EURL at least four times for each vial: before dispatch, just after dispatch, one week after dispatch (at the last time for start of analysis by the participants), and two weeks after dispatch. The tests were performed to check for possible matrix effects as well as the stability of the vials and matrix together.

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 10 numbered vials, each containing freeze-dried material with or without *Campylobacter* spp., and two plastic vacuum bags, each with about 60 g of shredded cabbage. The cabbage was to be divided into 10 g portions, one for each of the 10 vials. A Micro-T-Log was included in longer shipments 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 the remaining four NRLs within two days (Table 2).

The analysis was recommended to be started the same week as the PTs were dispatched from the EURL, and at the latest on the 15th 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 cabbage was recommended to be stored between 1 °C and 8 °C and the vials at −20 °C or lower until start of analysis. The dates for start of analysis are presented in Table 2.

Table 2. Dates of arrival and start of analysis of proficiency test No. 29, 2021.

Arrival	**Number of NRLs (N=33)**	**Start of analysis**	**Number of NRLs (N=33)**
9th of March	29	9th of March	2
10th of March	4	10th of March	10
		11th of March	4
		12th of March	2
		14th of March	1
		15th of March	13
		16th of March	1

Methods for analysis

The NRLs were recommended to follow ISO 10272-2:2017 for performing PT 29. However, if their standard laboratory procedure followed a different method, they were allowed to use that method for the test. Thirty-one 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 33 NRLs, 19 reported using commercial gas-generating kits, nine microaerobic incubators, six 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 sample, where results within median value ±2σMADe ($|z| \leq 2.0$) were given score 2, results between ±2σMADe and ±3σMADe ($2.0 < |z| < 3.0$) were given score 1 and results outside ±3σMADe ($|z| \geq 3.0$) were given score 0. 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.

When the −2σMADe and/or the −3σMADe limit fell below 1.0 log cfu/g, the minimum score given for results below this level, including results where no campylobacters were reported, was adjusted.

In cases when duplicate vials were used in the PT (sample No. 2 and 5, No. 1 and 8, and No. 3 and 4, 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 29 was distributed to 33 NRLs and all of them reported the results of the analysis. Nineteen laboratories started the analysis the same week the samples were dispatched from the EURL, and 14 NRLs the week after (Table 2).

Enumeration of *Campylobacter* spp. (mandatory)

Of the 33 NRLs, 25 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 21 negative results of samples with *Campylobacter* were reported (Figure 1 and Figure 2).

Generally, the median results in the PT for the *Campylobacter*-containing samples were lower than expected, based on the levels in the freeze-dried vials and the pre-tests with cabbage performed by the EURL. Vials with higher levels tended to decrease more than vials with lower levels, with the consequence that there was low variability in level between samples, and all median levels were quite low. The median values of the enumerations varied from 2.00 (sample No. 5) to 2.91 (sample No. 9) $\log_{10}$ cfu/g.

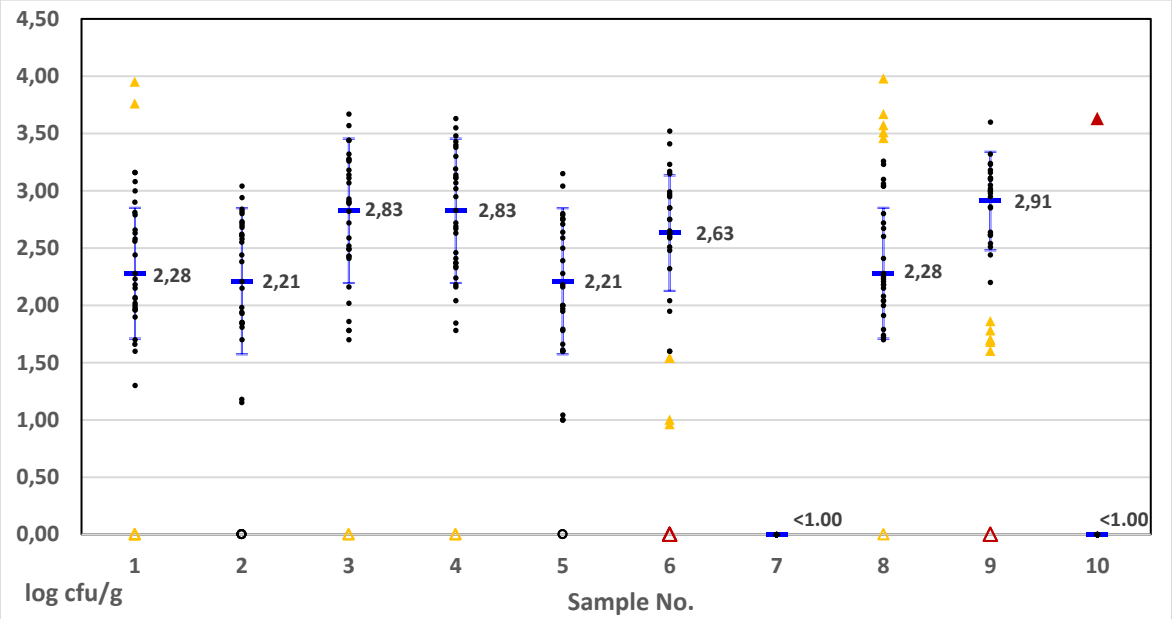

Figure 1. The number ($\log_{10}$ cfu/g) of *Campylobacter* spp. reported by 33 laboratories in PT 29 (2021). The samples reported as *Campylobacter* spp. not detected are shown as 0 in the figure and are represented by non-filled triangles (partly or unacceptable results) or circles (acceptable results). 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 (with some exceptions, see footnotes to Table 4) means that they fall outside the ±2σMADe and ±3σMADe limits, respectively.

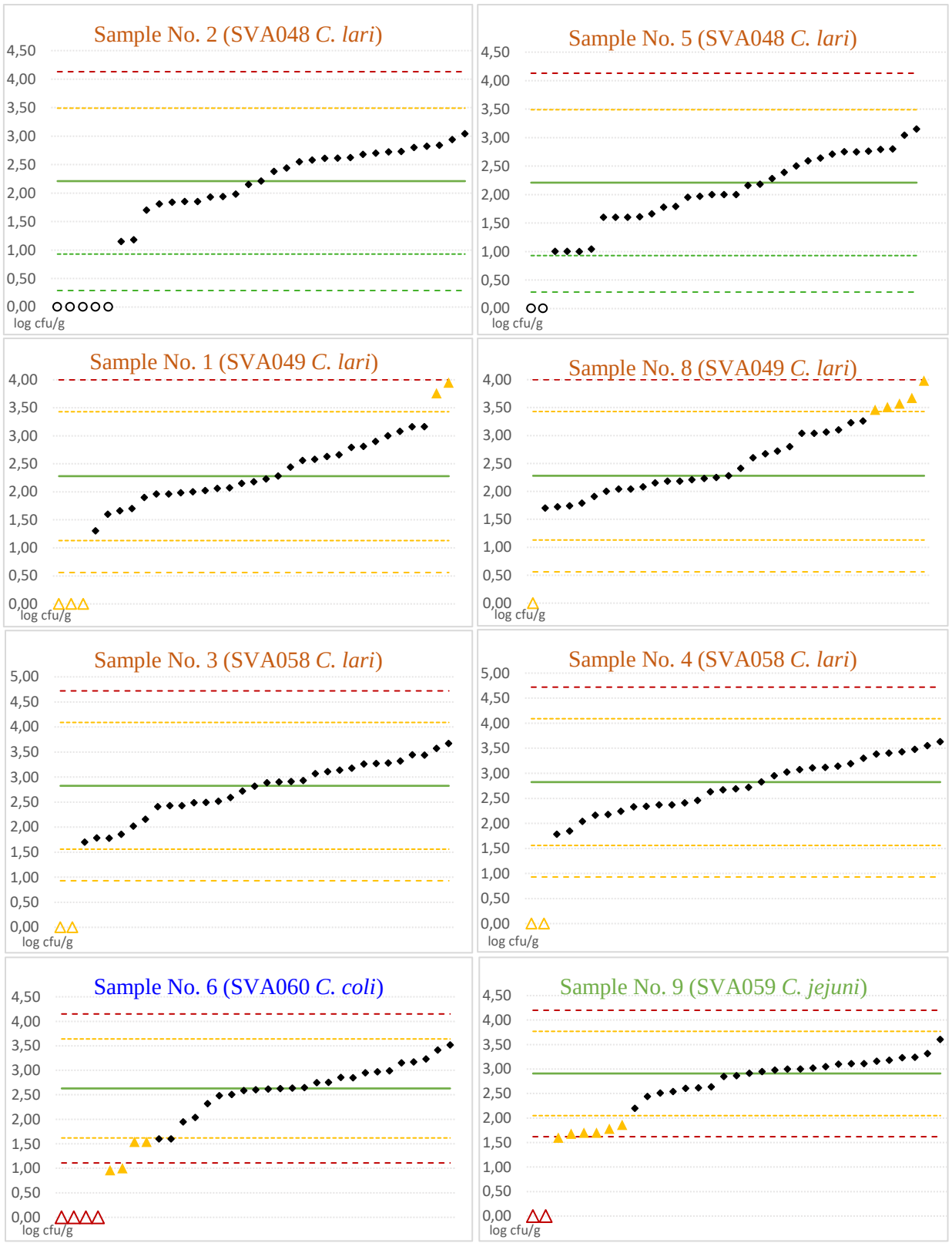

Figure 2. The number (log_{10} cfu/g) of *Campylobacter* spp. reported for each of the eight samples positive for *Campylobacter* by 33 laboratories in PT 29 (2021). Samples reported as *Campylobacter* spp. not detected (<1.00 log cfu/g) are shown as 0 in the figure and are represented by non-filled triangles (partly 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.

Because of the low levels, for some samples it could not be excluded that negative results (<1.00 $\log_{10}$ cfu) were proper results that occurred just by chance. Consequently, adjustments when the −2σMADe and/or the −3σMADe limit fell below 1.0 log cfu/g were made. Seven negative results (of sample No. 2 and 5) were considered as fully acceptable (given the score 2), eight negative results (of sample No. 1, 3, 4 and 8) as partly acceptable (given the score 1), and six negative results (of sample No. 6 and 9) as unacceptable (given the score 0).

The low variability in level between the three duplicate samples intended for the validation study (sample No. 2 and 5, No. 1 and 8, and No. 3 and 4, respectively) precluded the data from being usable for validation of the method.

Performance in enumeration of *Campylobacter* spp.

Despite the suboptimal stability during transport, the results were judged usable for evaluation of the NRLs' performance, after adjusting the scoring of negative results. The chosen method for assessment, which take the real variability between PT participants into account, implies that a higher variability is also reflected in wider acceptance ranges.

The results of using the five-level grading scale for the overall assessment of the NRLs' enumeration of *Campylobacter* spp. are presented in Table 3 and Figure 3.

According to the assessment, 31 NRLs (27 Member State NRLs, MS-NRLs) fulfilled the criterion for excellent or good performance and one MS-NRL scored below the acceptable limit (Table 3 and Figure 3). The overall median percentage of scores was 95% (50% Central Range (CR): 95.0%–100%).

The NRLs' enumeration results and z-scores for the eight samples positive for *Campylobacter* included in PT 29 are presented in Table 4.

Table 3. Overall performance of the NRLs' enumeration of *Campylobacter* spp. (n=33) in proficiency test No. 29 (2021).

Grade	**Scoring limits for each performance grade**	**Number (proportion) of NRLs with performance within scores**	
		All NRLs n=33	**MS-NRLs n=28**
Excellent	95.1–100%	14 (42%)	11 (39%)
Good	85.0–95.0%	17 (52%)	16 (57%)
Acceptable	70.0–84.9%	1 (3%)	0 (0%)
Needs improvement	57.0–69.9%	1 (3%)	1 (4%)
Poor	<57.0%	0 (0%)	0 (0%)

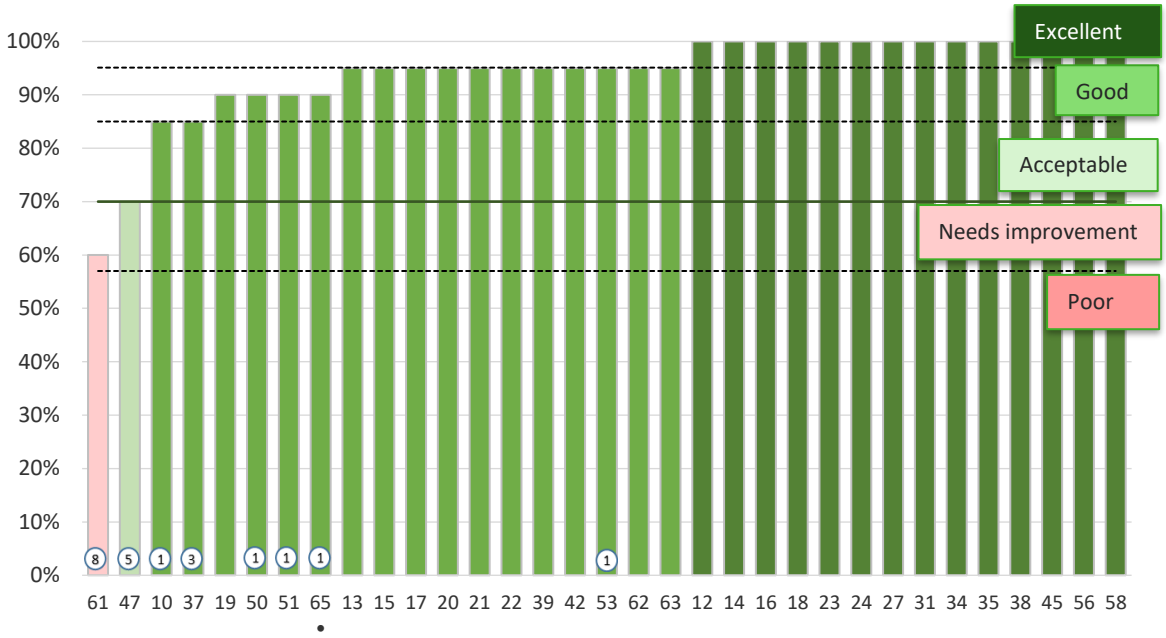

Figure 3. Distribution of the results of participating NRLs (n=33), represented by lab ID, in combined score for enumerations of eight samples with *Campylobacter* and two samples without *Campylobacter* in PT 29 (2021). Limits for grading of the overall performance are marked by horizontal lines. The numbers in white circles denote the number of negative results in samples with *Campylobacter*, and • denotes false positive results.

Table 4. Results from the enumeration and z-scores of samples with *Campylobacter* in proficiency test No. 29 (2021). 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. Green shadowed cells indicate that no campylobacters were detected, but the result was within the acceptable limits.

	Sample 1		**Sample 2**		**Sample 3**		**Sample 4**		**Sample 5**		**Sample 6**		**Sample 8**		**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	3.95	2.93	2.94	1.15	3.27	0.71	3.12	0.47	3.15	1.47	1.54	-2.16	3.98	2.98	3.23	0.74
12	1.96	-0.56	1.70	-0.80	2.41	-0.66	2.33	-0.79	2.00	-0.33	2.95	0.63	2.00	-0.49	2.98	0.16
13	2.81	0.93	2.61	0.63	3.67	1.34	3.40	0.91	3.04	1.30	3.17	1.07	3.67	2.44	2.44	-1.09
14	1.90	-0.67	2.38	0.27	2.93	0.17	3.14	0.50	2.71	0.78	1.95	-1.35	2.23	-0.09	2.86	-0.12
15	2.06	-0.39	1.18	-1.62	2.91	0.13	3.02	0.31	1.79	-0.66	2.64	0.02	2.21	-0.12	1.68	-2.86
16	2.56	0.49	2.82	0.96	3.44	0.98	3.48	1.04	2.80	0.93	2.63	0.00	2.18	-0.18	3.05	0.33
17	1.66	-1.09	<1.00	-1.90[a]	2.16	-1.06	2.37	-0.72	2.16	-0.08	2.75	0.24	1.91	-0.65	1.86	-2.44
18	2.44	0.28	2.73	0.82	3.07	0.39	3.55	1.15	2.76	0.86	2.75	0.24	2.41	0.23	3.11	0.47
19	2.66	0.67	2.70	0.77	3.44	0.98	3.07	0.39	2.75	0.85	1.54	-2.16[b]	3.06	1.37	1.70	-2.81
20	1.30	-1.72	1.93	-0.44	2.89	0.10	2.83	0.01	2.18	-0.05	1.00	-3.23	2.04	-0.42	2.64	-0.63
21	2.58	0.53	2.61	0.63	3.11	0.45	3.11	0.45	<1.60[c]	-0.96	2.65	0.04	2.28	0.00	<1.60[c]	-3.05[d]
22	2.07	-0.37	2.55	0.53	2.82	-0.01	2.46	-0.58	2.59	0.60	0.96	-3.31[b]	2.04	-0.42	3.24	0.77
23	1.98	-0.53	2.44	0.36	2.90	0.12	2.63	-0.31	1.00	-1.90	2.32	-0.61	3.10	1.44	3.02	0.26
24	3.00	1.26	1.81	-0.63	2.02	-1.28	2.16	-1.06	1.78	-0.67	<1.60[c]	-2.04[d]	3.04	1.33	2.51	-0.93
27	1.96	-0.56	1.15	-1.66	2.49	-0.53	2.95	0.20	2.39	0.28	2.51	-0.24	2.18	-0.18	2.95	0.09
31	3.08	1.40	2.84	0.99	3.32	0.79	3.38	0.88	2.75	0.85	2.04	-1.17	2.80	0.91	3.00	0.21
34	2.15	-0.23	1.85	-0.56	2.43	-0.63	2.72	-0.17	2.64	0.67	2.59	-0.08	3.04	1.33	2.54	-0.86
35	2.28	0.00	2.80	0.93	3.14	0.50	2.24	-0.93	2.50	0.45	3.41	1.55	2.25	-0.05	3.16	0.58
37	<1.00	-2.24[a]	<1.00	-1.90[a]	1.78	-1.66	2.18	-1.02	<1.00	-1.90[a]	2.60	-0.06	3.57	2.26	1.78	-2.63
38	2.00	-0.49	1.98	-0.36	2.43	-0.63	2.34	-0.77	2.00	-0.33	2.97	0.67	2.08	-0.35	3.00	0.21
39	2.79	0.89	2.68	0.74	3.18	0.56	3.19	0.58	2.79	0.91	3.15	1.03	3.46	2.07	3.32	0.95
42	2.23	-0.09	1.85	-0.56	1.78	-1.66	1.78	-1.66	1.00	-1.90	2.62	-0.02	2.72	0.77	1.70	-2.81
45	3.16	1.54	2.21	0.00[a]	2.49	-0.53	2.37	-0.72	1.66	-0.86	2.85	0.44[a]	1.74	-0.95	2.61	-0.70[a]
47	<1.00	-2.24[a]	<1.00	-1.90[a]	<1.00	-2.90[a]	1.85	-1.56	1.04	-1.83	<1.00	-3.23[a]	1.72	-0.97	<1.00	-4.44[a]
50	1.60	-1.19	1.84	-0.58	1.70	-1.79	2.04	-1.25	1.95	-0.41	<1.00	-3.23[a]	1.70	-1.02	2.20	-1.65
51	1.70	-1.02	2.62	0.64	2.52	-0.48	2.41	-0.66	<1.60[c]	-0.96	<1.00	-3.23[a]	1.79	-0.86	2.91	0.00
53	2.63	0.61	1.94	-0.42	1.86	-1.53	<1.00	-2.90[a]	1.97	-0.38	<1.60[c]	-2.04[d]	3.23	1.66	3.11	0.47
56	2.18	-0.18	2.15	-0.09	3.28	0.72	3.43	0.96	1.61	-0.94	2.48	-0.30	2.15	-0.23	2.62	-0.67
58	2.90	1.09	2.72	0.80	3.57	1.18	3.63	1.28	2.00	-0.33	3.52	1.77	2.67	0. 68	3.60	1.60
61	<1.00	-2.24[a]	<1.00	-1.90[a]	<1.00	-2.90[a]	<1.00	-2.90[a]	<1.00	-1.90[a]	<1.00	-3.23[a]	<1.00	-2.24[a]	<1.00	-4.44[a]
62	3.76	2.59	3.04	1.30	3.26	0.69	3.30	0.75	2.28	0.11	2.99	0.71	3.26	1.72	3.18	0.63
63	2.02	-0.46	2.58	0.58	2.59	-0.37	2.67	-0.25	<1.60[c]	-0.96	3.23	1.19	3.51	2.15	3.10	0.44
65	3.16	1.54	<1.00	-1.90[a]	2.72	-0.17	2.69	-0.21	1.00	-1.90	2.85	0.44	2.60	0.56	2.85	-0.14
Median[e]	**2.28**	2.26	**2.21**	2.50	**2.83**	2.89	**2.83**	2.72	**2.21**	2.00	2.63		**2.28**	2.35	2.91	
MADe	**0.39**	0.37	**0.43**	0.34	**0.43**	0.40	**0.43**	0.40	**0.43**	0.40	0.34		**0.39**	0.45	0.29	
σMADe	**0.57**	0.54	**0.64**	0.50	**0.63**	0.59	**0.63**	0.59	**0.64**	0.59	0.50		**0.57**	0.66	0.43	
±2σMADe	3.43	1.13	3.49	0.93	4.09	1.56	4.09	1.56	3.49	0.93	3.64	1.62	3.43	1.13	3.77	2.05
±3σMADe	4.00	0.56	4.13	0.29	4.72	0.93	4.72	0.93	4.13	0.29	4.15	1.11	4.00	0.56	4.20	1.62

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

[b] Reported results below 1.60 log_{10} cfu/g were given score 1 despite falling below the −3σMADe limit, since they correctly should have been reported as "present but lower than 1.60 log_{10} cfu/g", and then yielded score 2.

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

[d] Rounded to −2.0 or −3.0 and considered on the limit, not exceeding it.

[e] Median value of results for both samples of duplicate vials (No. 1 and 8, 2 and 5, and 3 and 4, 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)

Twenty-nine (88%) of the 33 NRLs reported results of species identification. Only one misidentification was reported, of sample No. 6 (Table 5). Twenty-one of the 29 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, 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.

Seventeen of the 29 NRLs reported that they used MALDI-TOF MS for the species identification, in four cases in combination with other techniques. Eleven 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). Other protocols reported to be used by more than one NRL were the PCR assays by Denis *et al.* (1999) and Best *et al.* (2003). Ten NRLs used biochemical tests (at least detection of catalase), in eight 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 nine NRLs combined two techniques for the species identification.

Table 5. Species identification reported by 29 NRLs in the voluntary part of proficiency test No. 29 (2021).

		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 lari*			27	2	
2.	*Campylobacter lari*			27	2	
3.	*Campylobacter lari*			28	1	
4.	*Campylobacter lari*			28	1	
5.	*Campylobacter lari*			28	1	
6.	*Campylobacter coli*		26	1	2	
7.	Negative				26	3
8.	*Campylobacter lari*			29		
9.	*Campylobacter jejuni*	29				
10.	*Escherichia coli*				4	25

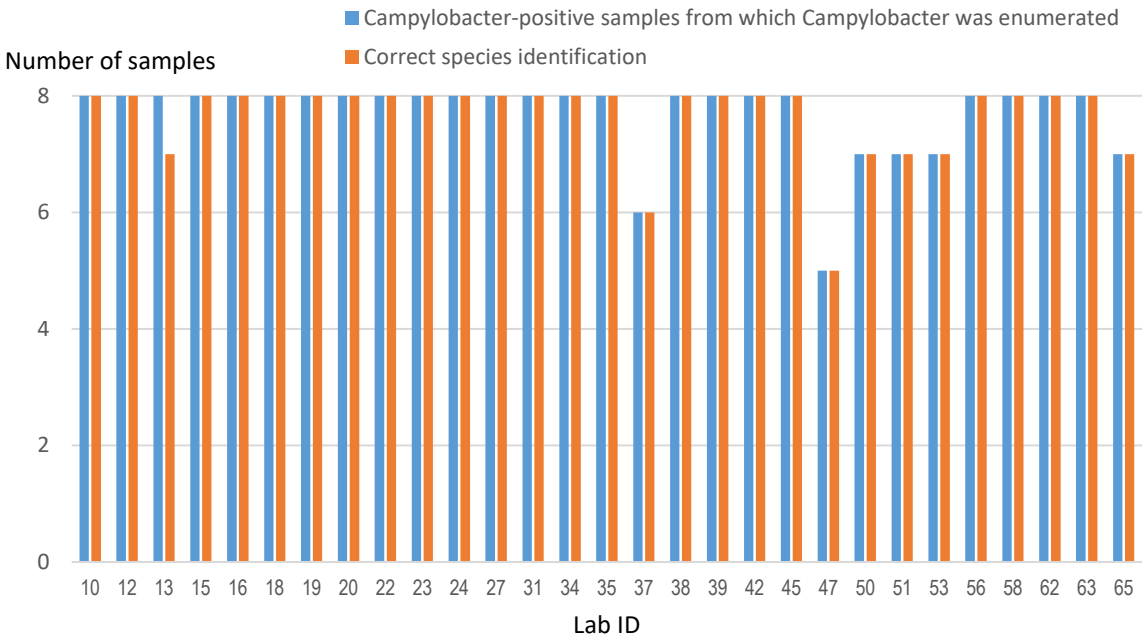

Figure 4. Results by 29 NRLs reporting results for species identification in the voluntary part of proficiency test No. 29 (2021).

Performance in identification of *Campylobacter* spp.

All 29 NRLs reporting results for species identification of *Campylobacter* fulfilled the criterion for excellent or good performance in identification of *Campylobacter* spp. (Table 6). The overall median sensitivity in correctly identifying *Campylobacter* spp. was 100% (50% CR: 100%–100%).

Table 6. Overall performance of NRLs' sensitivity in correctly identifying *Campylobacter* spp. in the voluntary part of PT 29 (2021).

		Identification of *Campylobacter* spp.	
Grade	**Sensitivity**	**Number of NRLs (%) All NRLs, n=29**	**Number of NRLs (%) MS-NRLs, n=28**
Excellent	95.1–100%	28 (97%)	27 (96%)
Good	85.0–95.0%	1 (3%)	1 (4%)
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%)

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