



## **EURL-CAMPYLOBACTER**

### **REPORT**

## **PROFICIENCY TEST NUMBER 26**

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**Enumeration (and voluntary species identification) of  
*Campylobacter***

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## Abbreviations

C.	<i>Campylobacter</i>
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 26, 2020

The EU reference laboratory for *Campylobacter* organised proficiency test (PT) number 26 on enumeration of *Campylobacter* spp. in chicken skin in March 2020. The PT included enumeration of *Campylobacter* spp. in 10 chicken skin samples 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 26.

Thirty-eight NRLs in 27 EU Member States and in the United Kingdom, Iceland, Norway, Switzerland and Albania had registered for and received the PT, and 33 NRLs reported (at least partly) results within the specified timeframe. Due to the Covid-19 pandemic, the last day to start the analysis was postponed four weeks and the last day to submit the results was postponed three weeks.

Thirty-two of the 33 participating NRLs used the recommended method ISO 10272-2:2017 for analysing the samples. Twenty-nine (88%) fulfilled the criterion for excellent or good performance in enumeration of *Campylobacter* spp. which is about the same level as the four previous years. No NRL scored below the acceptable limit.

Twenty-six (79%) of the 33 NRLs reported results of species identification of *Campylobacter*, which is a somewhat lower proportion than previous years. However, the results were very good: 25 NRLs (96%) fulfilled the criterion for excellent or good performance in identification of *Campylobacter* spp., and none scored below the acceptable limit.

Although the Covid-19 pandemic prevented five of 38 of the NRLs (13%) from performing PT 26 in time, the participating NRLs reported high-level results. The majority of the NRLs met the criteria for excellent or good performance in both enumeration and species identification, and none scored below the acceptable limit in any of the tests. Thus, the *Campylobacter* NRLs are well meeting the requirements of being NRLs.

## Introduction

Proficiency test (PT) number 26 on enumeration of *Campylobacter* spp. in chicken skin was organised by the EU reference laboratory (EURL) for *Campylobacter* in March 2020. Thirty-eight national reference laboratories (NRLs) in 27 EU Member States (some Member States have more than one NRL) and in the United Kingdom, Iceland, Norway, Switzerland, and Albania had registered for and received the PT. Due to the Covid-19 pandemic, the last day to start the analysis was postponed four weeks and the last day to submit the results was postponed three weeks. Still, five laboratories were unable to carry out the PT as anticipated. This report only includes the results generated and reported before each deadline. Thirty-three NRLs reported results, out of which one NRL reported results for enumeration, but not from the confirmatory tests.

Thirty-two of the 33 NRLs reported that they were accredited for detection of *Campylobacter* and 27 were also accredited for enumeration of *Campylobacter*.

The PT included enumeration of *Campylobacter* spp. in 10 chicken skin samples 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 26.

Table 1. Contents of the 10 vials distributed to the NRLs in proficiency test No. 26 (2020).

Sample No.	Species	Level <sup>b</sup> (log <sub>10</sub> cfu/vial)		Batch No.
1	<i>Campylobacter coli</i>	3.90		SLV334
2	<i>Campylobacter jejuni</i> <sup>a</sup>	4.82		SLV305
3	<i>Campylobacter jejuni</i> and <i>Escherichia coli</i>	3.53	4.00	SLV313
4	<i>Escherichia coli</i>		3.41	SLV159
5	<i>Campylobacter jejuni</i> <sup>a</sup>	4.60		SLV336
6	<i>Campylobacter lari</i>	5.10		SLV335
7	<i>Campylobacter jejuni</i> <sup>a</sup>	3.71		SLV306
8	<i>Campylobacter coli</i>	5.50		SLV333
9	<i>Campylobacter jejuni</i> <sup>a</sup>	6.12		SVA038
10	Negative			SLV289/SLV272

<sup>a</sup> All *Campylobacter jejuni* strains were hippurate positive.

<sup>b</sup> According to the producer.

## 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 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 six months. The broilers were slaughtered at a slaughterhouse with a very low level of *Campylobacter*-positive flocks (2.9 % during 2019) and no positive flocks at all for three months before taking out and sending thigh skin to the EURL. Chicken skin and caecal samples from the broiler flock tested negative for presence of *Campylobacter*. The chicken skin was freeze-stored until distribution of the PT.

### Production and quality control of the vials

The vials with freeze-dried bacterial cultures used in the PT were produced and tested for stability and homogeneity by the Swedish Food Agency or 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 tested vials in a batch was 0.50  $\log_{10}$  cfu.

Also, enumeration of *Campylobacter* spp. in chicken skin according to ISO 10272-2:2017 was performed by the EURL four times for each batch: before dispatch, just after dispatch, two weeks, and six weeks after dispatch, i.e. at the last time for start of the analysis by the participants. The last test was included after the decision to postpone the last day for start of analysis and report. 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 9<sup>th</sup> of March, 2020. 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 bottle with chicken meat (ca 120 g), to be divided into 10 g portions, one for each of the 10 vials. A Micro-T-Log was included in each shipment to record the temperature every second hour during transport.

Twenty-nine of 36 reporting NRLs received the PT within one day after the packages had been dispatched from the EURL, five NRLs two days, and one NRL four days after (Table 2).

The analysis was recommended to be started the same week as the PTs were dispatched from the EURL. 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. If the analysis could not be started the same week, the vials were recommended to be stored at -70 °C and the chicken skin at -20 °C until the analysis could be started, at the latest 23<sup>rd</sup> of March according to the initial instructions, which also set the last day to report the results to 20<sup>th</sup> of April, 2020.

Due to the Covid-19 outbreak, both the last day to start the analysis and to report the results were postponed by four and three weeks, to 20<sup>th</sup> of April and 11<sup>th</sup> of May, respectively. The aim was to give as many NRLs as possible the opportunity to perform the PT despite the extraordinary circumstances, and to have their results and performance included in the normal analysis and test report. The postponement decision was taken and communicated by e-mail 19<sup>th</sup> of March. In spite of this announcement, the NRLs were encouraged to perform the test as soon as possible, and to report their results without unnecessary delay.

The dates for the start of analysis are presented in Table 2. Totally, four NRLs made use of the extended deadline for start of the analysis, including one NRL receiving a new test sent out 6<sup>th</sup> of April due to technical problems with storage. Neither the additional tests performed by the EURL nor the results of the PT as a whole gave indications of any detrimental effect on the results, e.g. higher variability, of the extended deadline. Therefore, no adjustments in the performance assessment were made because of this.

Table 2. Dates of arrival and start of the analysis of proficiency test No. 26, 2020.

<b>Arrival</b>	<b>Number of NRLs (N=36<sup>a</sup>)</b>	<b>Start of analysis</b>	<b>Number of NRLs (N=33)</b>
10 <sup>th</sup> of March	29	10 <sup>th</sup> of March	5
11 <sup>th</sup> of March	5	11 <sup>th</sup> of March	6
13 <sup>th</sup> of March	1	12 <sup>th</sup> of March	3
7 <sup>th</sup> of April <sup>b</sup>	1	13 <sup>th</sup> of March	1
		16 <sup>th</sup> of March	9
		17 <sup>th</sup> of March	2
		18 <sup>th</sup> of March	1
		23 <sup>rd</sup> of March	2
		7 <sup>th</sup> of April <sup>b</sup>	1
		20 <sup>th</sup> of April	3

<sup>a</sup>The PT was distributed to 38 NRLs.

<sup>b</sup>Due to technical problems related to the Covid-19 outbreak, one NRL received a new test sent out 6<sup>th</sup> of April.

## Methods for analysis

The NRLs were recommended to follow ISO 10272-2:2017 for performing PT 26. 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 one NRL an internal method.

*Campylobacter* spp. should be incubated in a microaerobic atmosphere, with oxygen content of  $5\% \pm 2\%$ , and carbon dioxide  $10\% \pm 3\%$ . 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, 11 microaerobic incubators, four the Anoxomat® system and three other methods (jars filled with gas mixture, 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  $\pm 2\sigma$ MADe ( $|z| \leq 2.0$ ) were given score 2, results between  $\pm 2\sigma$ MADe and  $\pm 3\sigma$ MADe ( $2.0 < |z| < 3.0$ ) were given score 1 and results outside  $\pm 3\sigma$ MADe ( $|z| \geq 3.0$ ) 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.

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  $\pm 2\sigma$ MADe 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”.

For sample No. 3, which resulted in the most homogenous results, a result of within  $0.5 \log_{10}$  units of the participants’ median value was determined to be acceptable (given the maximum score 2) following the  $0.5 \log_{10}$  rule (ISO 22117:2019).

## Assessment of performance in identification

The performance in correctly identifying the species for the samples where *Campylobacter* was detected, the sensitivity, was categorized 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 26 was distributed to 38 NRLs and 33 reported the results of the analysis within the time limits set. Fifteen laboratories started the analysis the same week the samples were dispatched from the EURL, twelve NRLs the week after, two NRLs two weeks after, one NRL four weeks after (a new test sent out the same week, but of the same batch as the other tests so had been stored by the EURL for four weeks), and three NRLs six weeks after (Table 2).

### Enumeration of *Campylobacter* spp. (mandatory)

Of the 33 NRLs, 28 correctly reported *Campylobacter* spp. in all samples where *Campylobacter* spp. were included and no detection of *Campylobacter* in the samples without *Campylobacter*. No false positive results, but five false negative results, of sample No. 1, 6 and 9, were reported. The median values of the enumerations varied from 2.44 (sample No. 1) to 4.09 (sample No. 9) log<sub>10</sub> cfu/g (Figure 1 and Figure 2).

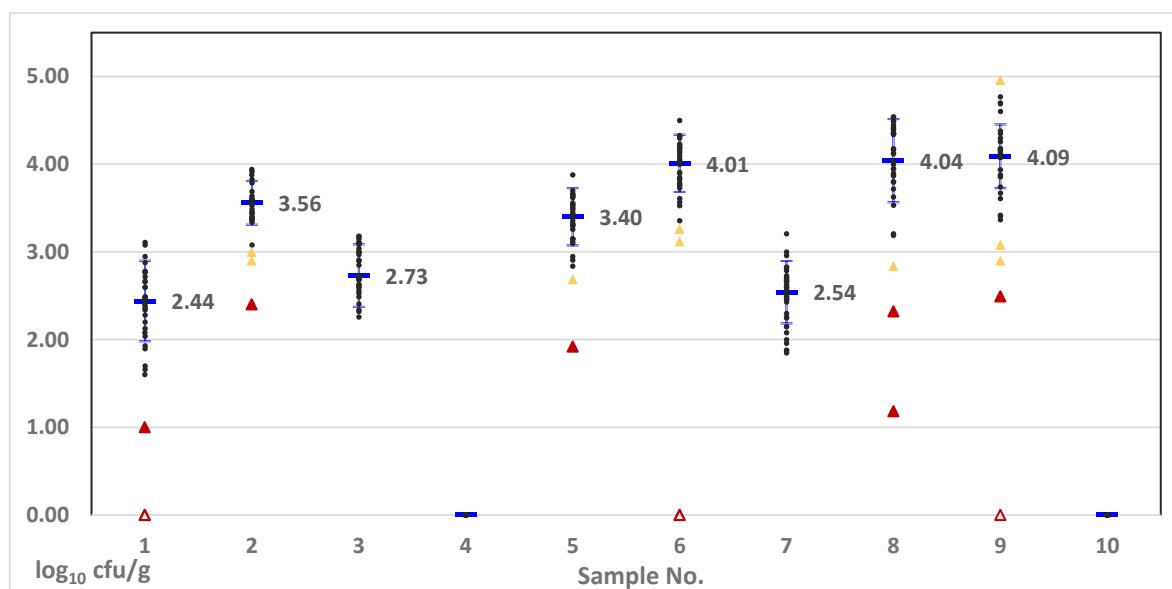


Figure 1. The number (log<sub>10</sub> cfu/g) of *Campylobacter* spp. reported by 33 laboratories in PT 26 (2020). The samples reported as *Campylobacter* spp. not detected are shown as 0 in the figure. The median values are displayed in numbers and marked with horizontal lines. Vertical bars show the σMADe. Values outside the ± 2σMADe and ± 3σMADe limits are shown as small and large triangles, respectively.

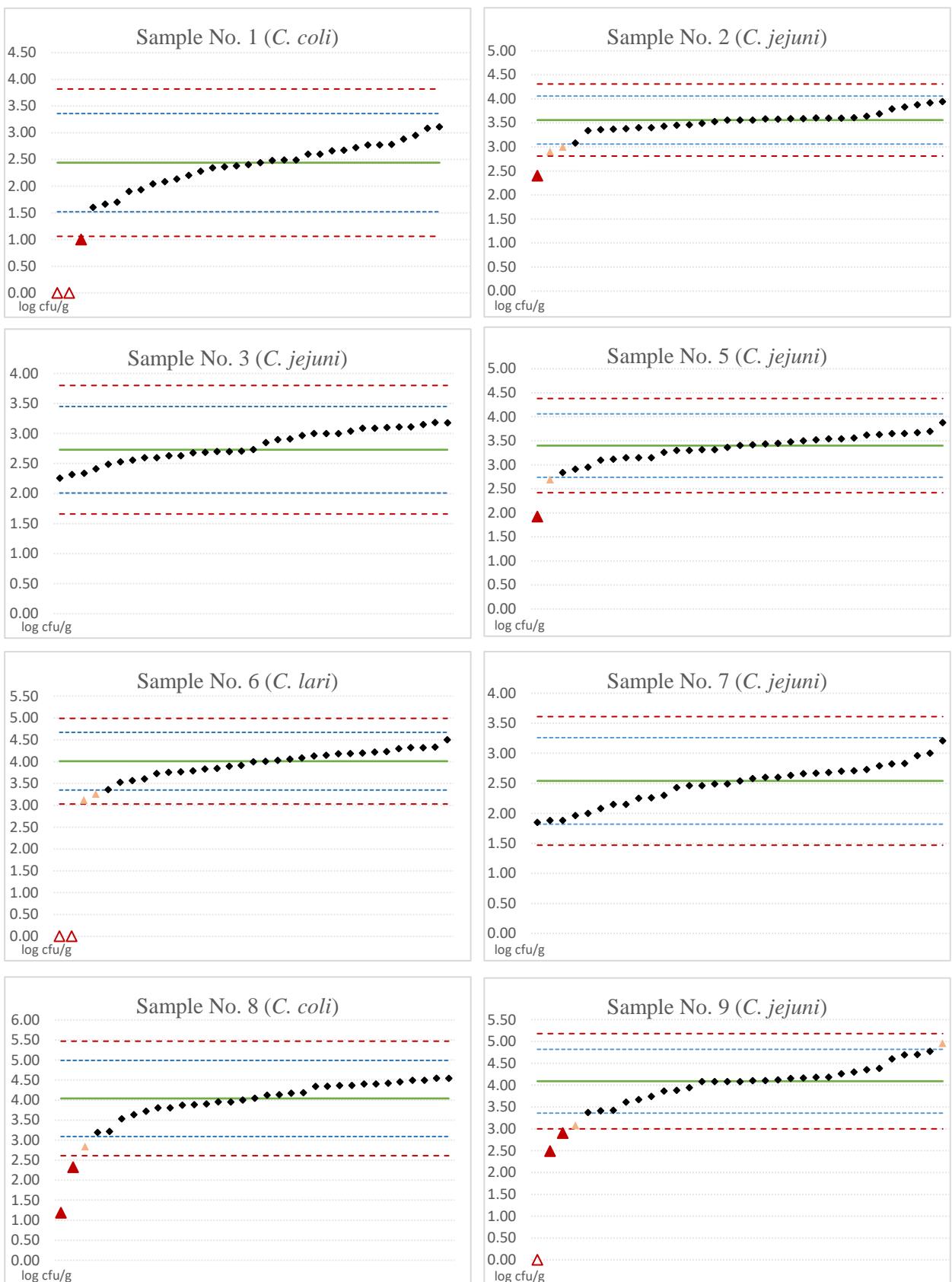


Figure 2. The number ( $\log_{10}$  cfu/g) of *Campylobacter* spp. reported for each of the eight samples positive for *Campylobacter* by 33 laboratories in PT 26 (2020). Samples reported as *Campylobacter* spp. not detected are shown as 0 in the figure. The median values and the  $\pm 2\sigma$ MADe and  $\pm 3\sigma$ MADe limits are shown as horizontal lines. Values outside any of the limits are shown as 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 3 and Figure 3.

According to the assessment, 29 NRLs (25 Member State NRLs, MS-NRLs) fulfilled the criterion for excellent or good performance and no NRL scored below the acceptable limit (Table 3 and Figure 3). The overall median percentage of scores was 100% (50% Central Range (CR): 95.0%–100%).

The NRLs' enumeration results and z-scores for the eight samples positive for *Campylobacter* included in PT 26 are presented in Table 4.

Table 3. Overall performance of the NRLs' enumeration of *Campylobacter* spp. (n=33) in proficiency test No. 26 (2020).

Grade	Scoring limits for each performance grade	Number (proportion) of NRLs with performance within scores	
		All NRLs n=33	MS-NRLs n=26
Excellent	95.1–100%	22 (67%)	20 (77%)
Good	85.0–95.0%	7 (21%)	5 (19%)
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%)

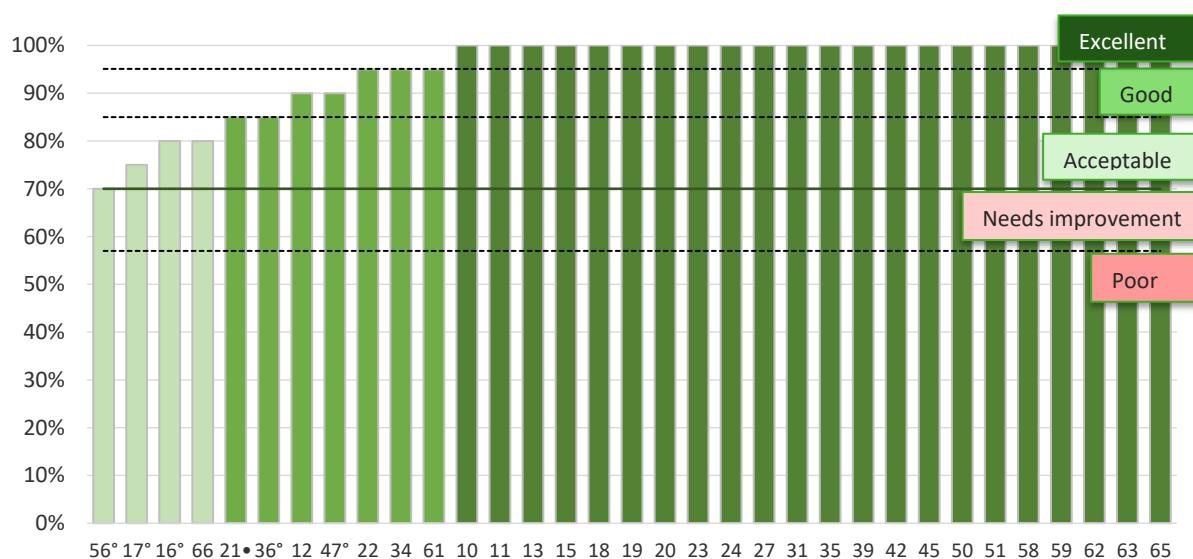


Figure 3. Distribution of the results of participating NRLs (n=33), represented by lab ID, in combined score for enumerations of eight samples with *Campylobacter* and two samples without *Campylobacter* in PT 26 (2020). Limits for grading of the overall performance are marked by horizontal lines. Each ° represents a false negative result and • for not confirmed results.

Table 4. Results from the enumeration and z-scores of samples with *Campylobacter* in proficiency test No. 26 (2020). Yellow shadowed cells indicate values outside median values  $\pm 2\sigma$ MADe and z-scores  $\pm 2.0$ . Red shadowed cells indicate values outside median values  $\pm 3\sigma$ MADe and z-scores  $\pm 3.0$ .

	Sample 1		Sample 2		Sample 3		Sample 5		Sample 6		Sample 7		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
<b>10</b>	2.77	0.72	3.45	-0.44	2.73	0.00	3.36	-0.12	4.33	0.98	2.66	0.34	4.45	0.86	4.35	0.72
<b>11</b>	2.66	0.48	3.58	0.08	3.18	1.26	3.54	0.43	4.32	0.95	2.63	0.25	4.54	1.05	4.1	0.03
<b>12</b>	1.00 <sup>a</sup>	-3.13	3.60	0.16	3.00	0.76	3.52	0.37	3.83	-0.55	1.96	-1.63	3.95	-0.19	4.16	0.19
<b>13</b>	2.78	0.74	3.94	1.52	2.70	-0.08	3.88	1.47	4.50	1.50	3.21	1.88	4.36	0.67	4.77	1.87
<b>15</b>	2.67	0.50	3.69	0.52	3.18	1.26	3.63	0.71	4.20	0.58	2.30	-0.67	4.34	0.63	4.70	1.68
<b>16</b>	<1.00	-3.13 <sup>b</sup>	3.40	-0.64	2.53	-0.56	2.91	-1.50	3.61	-1.23	2.46	-0.22	1.18	-6.03	3.37	-1.98
<b>17</b>	<1.00	-3.13 <sup>b</sup>	3.08	-1.92	2.26	-1.32	2.95	-1.38	3.53	-1.47	2.49	-0.14	2.84	-2.53	2.49	-4.40
<b>18</b>	2.49	0.11	3.60	0.16	3.10	1.04	3.54	0.43	4.30	0.89	2.82	0.79	4.42	0.80	4.69	1.65
<b>19</b>	2.28	-0.35	3.53	-0.12	2.63	-0.28	3.65	0.77	4.23	0.67	2.96	1.18	4.13	0.19	4.38	0.80
<b>20</b>	1.90	-1.17	3.60	0.16	2.90	0.48	3.30	-0.31	3.90	-0.34	2.60	0.17	4.40	0.76	4.10	0.03
<b>21</b>	2.36 <sup>c</sup>	-0.17	3.00 <sup>c</sup>	-2.24	3.11 <sup>c</sup>	1.07	3.48 <sup>c</sup>	0.25	3.73 <sup>c</sup>	-0.86	1.88 <sup>c</sup>	-1.85	3.87 <sup>c</sup>	-0.36	2.90 <sup>c</sup>	-3.28
<b>22</b>	2.20	-0.52	2.90	-2.64	2.70	-0.08	3.10	-0.92	3.57	-1.35	1.85	-1.94	3.95	-0.19	3.42	-1.84
<b>23</b>	2.13	-0.67	3.37	-0.76	2.71	-0.06	3.32	-0.25	3.36	-1.99	2.67	0.37	3.19	-1.79	4.15	0.17
<b>24</b>	2.49	0.11	3.59	0.12	2.68	-0.14	3.45	0.15	3.85	-0.49	2.54	0.00	4.49	0.95	4.08	-0.03
<b>27</b>	2.48	0.09	3.46	-0.40	2.97	0.67	3.42	0.06	4.01	0.00	2.68	0.39	3.80	-0.51	4.08	-0.03
<b>31</b>	2.77	0.72	3.64	0.32	3.04	0.87	3.40	0.87	4.15	0.43	2.49	-0.14	4.40	0.76	4.18	0.25
<b>34</b>	2.60	0.35	3.92	1.44	2.60	-0.37	3.62	0.67	4.32	0.95	3.00	1.29	3.72	-0.67	4.96	2.40
<b>35</b>	2.34	-0.22	3.49	-0.28	2.34	-1.10	3.70	0.92	4.13	0.37	2.71	0.48	4.54	1.05	3.86	-0.63
<b>36</b>	2.40	-0.09	3.59	0.12	2.56	-0.48	3.50	0.31	3.12	-2.73	2.79	0.70	3.21	-1.75	<1.00	-8.51 <sup>b</sup>
<b>39</b>	2.88	0.96	3.61	0.20	3.09	1.01	3.56	0.49	4.22	0.64	2.73	0.53	4.36	0.67	3.94	-0.41
<b>42</b>	3.08	1.39	3.58	0.08	2.69	-0.11	2.84	-1.72	4.03	0.06	2.43	-0.31	4.49	0.95	4.30	0.58
<b>45</b>	1.66	-1.70	3.56	0.00	3.00	0.76	3.30	-0.31	4.06	0.15 <sup>b</sup>	2.83	0.82	4.12	0.17	4.08	-0.03
<b>47</b>	2.95	1.11	3.38	-0.72	3.09	1.01	3.15	-0.77	<1.00	-9.23	2.58	0.11	3.88	-0.34	3.41	-1.87
<b>50</b>	2.72	0.61	3.56	0.00	2.91	0.51	3.43	0.09	4.19	0.55	1.88	-1.85	4.17	0.27	4.08	-0.03
<b>51</b>	1.70	-1.61	3.79	0.92	3.00	0.76	3.67	0.83	3.76	-0.77 <sup>b</sup>	2.26	-0.79	3.80	-0.51	3.88	-0.58
<b>56</b>	1.93	-1.11	2.40	-4.64	2.41	-0.90	1.92	-4.54	<1.00	-9.23	2.00	-1.52	4.04	0.00	4.26	0.47
<b>58</b>	2.38	-0.13	3.88	1.28	3.11	1.07	3.15	-0.77	3.91	-0.31	2.15	-1.10	4.34	0.63	4.60	1.40
<b>59</b>	2.04	-0.87	3.40	-0.64	2.32	-1.15	3.15	-0.77	3.77	-0.74	2.60	0.17	3.90	-0.30	4.18	0.25
<b>61</b>	2.08	-0.78	3.43	-0.52	2.63	-0.28	3.32	-0.25	3.26	-2.30	2.15	-1.10	3.53	-1.07	3.67	-1.16
<b>62</b>	2.60	0.35	3.83	1.08	2.60	-0.37	3.65	0.77	4.18	0.52	2.70	0.45	4.00	-0.08	3.61	-1.32
<b>63</b>	3.11	1.46	3.56	0.00	3.15	1.18	3.12	-0.86	4.09	0.25	2.25	-0.82	4.18	0.30	4.12	0.08
<b>65</b>	2.44	0.00	3.34	-0.88	2.85	0.34	3.26	-0.43	4.00	-0.03	2.46	-0.22	3.63	-0.86	3.74	-0.96
<b>66</b>	1.60	-1.83	3.36	-0.80	2.49	-0.67	2.69	-2.18	3.79	-0.67	2.08	-1.29	2.32	-3.63	3.08	-2.78
Median	2.44		3.56		2.73		3.40		4.01		2.54		4.04		4.09	
MADe	0.31		0.13		0.24		0.22		0.22		0.24		0.32		0.25	
$\sigma$ MADe	0.46		0.25		0.36		0.33		0.33		0.36		0.47		0.36	
$\pm 2\sigma$ MADe	3.36	1.52	4.06	3.06	3.45	2.01	4.06	2.74	4.67	3.35	3.26	3.61	4.99	3.09	4.82	3.36
$\pm 3\sigma$ MADe	3.82	1.06	4.31	2.81	3.80	1.66	4.38	2.42	4.99	3.03	3.61	1.47	5.47	2.61	5.18	3.00

<sup>a</sup> Reported as **present** but lower than this value, calculations based on this value.

<sup>b</sup> Calculated from 1.00  $\log_{10}$  cfu/g.

<sup>c</sup> Not confirmed result.

## Species identification of *Campylobacter* spp. (voluntary)

Twenty-six (79%) of the 33 NRLs reported results of species identification (Table 5). Of the eight samples containing *Campylobacter*, four (sample No. 2, 3, 5, and 7) were correctly identified by all 26 NRLs.

Table 5. Species identification reported by 26 NRLs in the voluntary part of proficiency test No. 26 (2020).

Content of sample (vial)	Number of NRLs reporting				
	<i>Campylobacter jejuni</i>	<i>Campylobacter coli</i>	<i>Campylobacter lari</i>	<i>Campylobacter</i> spp. but unable to identify species	Other/No growth
1. <i>Campylobacter coli</i>	25				1
2. <i>Campylobacter jejuni</i>	26				
3. <i>Campylobacter jejuni</i> & <i>Escherichia coli</i>	26				
4. <i>Escherichia coli</i>				26	
5. <i>Campylobacter jejuni</i>	26				
6. <i>Campylobacter lari</i>			24	1	1
7. <i>Campylobacter jejuni</i>	26				
8. <i>Campylobacter coli</i>		1	25		
9. <i>Campylobacter jejuni</i>	24	1			1
10. Negative				26	

The isolated *Campylobacter* spp. were identified by biochemical tests and/or molecular methods, 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 nalidixic acid and cephalotin, and hydrogen sulphide production in triple sugar iron medium.

Twelve of the 26 NRLs reported that they used MALDI-TOF MS for the species identification, in two cases in combination with other techniques. Ten NRLs used one or more PCR assays, in two cases in combination with other techniques. Five NRLs reported to have used the multiplex PCR assay published by Wang et al. (2002), and two NRLs used the PCR protocol by Denis et al. (1999). Eight NRLs used biochemical tests (at least detection of catalase), in four cases in combination with MALDI-TOF MS or PCR.

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

## Performance in identification of *Campylobacter* spp.

Of the 26 NRLs reporting results for species identification of *Campylobacter*, 25 fulfilled the criterion for excellent or good performance in identification of *Campylobacter* spp., and none scored below the acceptable limit (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 26 (2020).

Identification of <i>Campylobacter</i> spp.			
Grade	Sensitivity	Number of NRLs (%) All NRLs, n=26	Number of NRLs (%) MS-NRLs, n=21
Excellent	95.1–100%	23 (88%)	19 (90%)
Good	85.0–95.0%	2 (8%)	1 (5%)
Acceptable	70.0–84.9%	1 (4%)	1 (4%)
Needs improvement	57.0–69.9%	0 (0%)	0 (0%)
Poor	<57.0%	0 (0%)	0 (0%)

## References

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