EURL-*Campylobacter* Proficiency Test Report

PT 39. Enumeration (and voluntary species identification) of *Campylobacter*

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PT 39. Enumeration (and voluntary species identification) of *Campylobacter*

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Cover image Enumeration of *Campylobacter* on mCCD agar. Photo: Ida Olsson/SVA.

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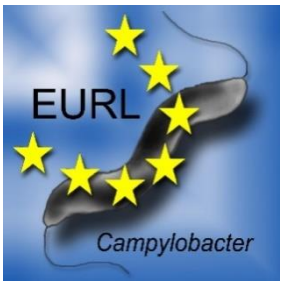
The European Commission officially designated the Swedish Veterinary Agency as the European Union reference laboratory (EURL) for *Campylobacter* on July 1st, 2006. The EURL regularly organises proficiency tests (PTs) for the national reference laboratories (NRLs) on methods of laboratory analysis for *Campylobacter* in different matrices of food or animal origin.



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Summary

The EU reference laboratory for *Campylobacter* organised proficiency test (PT) number 39 on enumeration of *Campylobacter* spp. in chicken skin in March 2025. 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) in enumeration of *Campylobacter* in chicken skin. Species identification of detected *Campylobacter* was a voluntary part of PT 39.

Participation in PT 39 was mandatory for at least one NRL per Member State (MS). Thirty-five NRLs in 27 EU MSs (some MSs have more than one NRL) and in five non-EU countries 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, and two NRLs used other methods.

Twenty-nine NRLs (83%) fulfilled the criterion for excellent or good performance in enumeration of *Campylobacter* spp., and three NRLs (one MS-NRL) scored below the acceptable limit. Thirty of the 35 NRLs reported results of species identification of *Campylobacter*, and 28 of them fulfilled the criterion for excellent performance in identification of *Campylobacter* spp. One MS-NRL scored below the acceptable limit.

In summary, the majority of the NRLs met the criteria for excellent or good performance in enumeration and species identification. Three NRLs and one NRL scored below the acceptable limits in enumeration and species identification, respectively.

Contents

Abbreviations

C.	*Campylobacter*
cfu	colony forming units
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
SD	standard deviation
spp.	species

Introduction

Proficiency test (PT) No. 39 on enumeration of *Campylobacter* spp. in chicken skin was organised by the EU reference laboratory (EURL) for *Campylobacter* in March 2025. Thirty-five national reference laboratories (NRLs) in 27 EU Member States (MSs, some MSs have more than one NRL) and in five non-EU countries received the PT. All 35 NRLs reported the test results and operational details to the EURL.

Thirty-two NRLs reported that they were accredited for detection of *Campylobacter* and 31 that they were accredited for enumeration of *Campylobacter*. Three NRLs were accredited for detection only, two NRLs were accredited for enumeration only, and one NRL was accredited neither for detection nor enumeration of *Campylobacter*.

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 in enumeration of *Campylobacter* spp. in chicken skin. Species identification of *Campylobacter* was a voluntary part of PT 39.

TABLE 1. Contents of the ten vials distributed to the NRLs in proficiency test No. 39, 2025.

Sample No.	Species	Level[b] (log_{10} cfu/vial)	Standard deviation[b] (log_{10} cfu)	Batch No.
1	*Campylobacter coli*	4.77	0.05	SLV367
2	*Campylobacter jejuni*[a]	3.24	0.04	SLV403
3	*Escherichia coli*	4.80	0.07	SVA061
4	*Campylobacter coli*	4.77	0.05	SLV367
5	*Campylobacter coli*	4.77	0.05	SLV367
6	Negative			
7	*Campylobacter jejuni*[a]	3.95	0.05	SLV401
8	*Campylobacter jejuni*[a]	3.95	0.05	SLV401
9	*Campylobacter coli*	5.04	0.06	SLV375
10	*Campylobacter jejuni*[a]	3.24	0.04	SLV403

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

[b] According to homogeneity test of ten vials after the production, or five vials in follow-up stability testing. The homogeneity and stability were evaluated according to ISO 33405:2024.

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 during winter season from a broiler producer that had not delivered any *Campylobacter*-positive flocks to slaughter during the preceding autumn (for more than five months). The broilers were slaughtered at a slaughterhouse with a history of low level of *Campylobacter*-positive flocks (5.1% during 2024).

On arrival chicken skin was tested in triplicate for detection after enrichment in Bolton and Preston broth. Each enrichment was streaked on modified charcoal cefoperazone deoxycholate (mCCD) and Butzler agar. The chicken skin tested negative for detection of *Campylobacter* by enrichment, but a moderate background flora was present. The chicken skin was packed in separate zip bags of about 120 g each and freeze-stored at –20 °C until distribution of the PT. In addition, chicken caecal material from the same chicken flock tested negative for *Campylobacter* by direct streak on mCCD and Butzler agar and by enrichment with Bolton broth.

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 according to ISO 33405:2024 by the producer. The standard deviation (SD) from the homogeneity testing of ten vials or the last follow-up stability testing of five vials analysed in repeatable conditions is included in Table 1. Before selecting vials for the PT, the EURL tested three vials of each batch containing *Campylobacter* spp. 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 (the same batch as in 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 (the PT content packed in a styrofoam box with freezing blocks and stored at room temperature for 24 h) and “worst case” transport conditions (the PT content packed in a styrofoam box with freezing blocks and stored at room temperature for 48 h). The test was also performed two weeks after dispatch at the last date for start of analysis by the participants, on vials first stored in “worst case” conditions (styrofoam box stored at room temperature for 48 h) before storage at −20 °C until start of analysis.

TABLE 2. Outline of stability testing under transport conditions for proficiency test No. 39, 2025.

Test occasion	Storage condition[a]	Number of samples tested[b]
Before dispatch	Best case	Each vial batch with *Campylobacter* × 2
Before dispatch	Worst case	Each vial batch with *Campylobacter* × 2
Two weeks after dispatch	Worst case	Each vial batch with *Campylobacter* × 2

[a] Best case transport conditions: The PT content packed in a styrofoam box with freezing blocks and stored in room temperature for 24 h and worst case transport conditions: The PT content packed in a styrofoam box with freezing blocks and stored in room temperature for 48 h.
[b] Enumeration of *Campylobacter* spp. in chicken skin according to ISO 10272-2:2017.

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 batches) and transport (both "best case" and "worst case") conditions was therefore evaluated per used vial batch (in total six vials tested of each *Campylobacter*-containing batch according to Table 2). The variation observed (the highest range was 0.80 $\log_{10}$ cfu with a SD of 0.26 $\log_{10}$ cfu for SLV367) was accounted the variability of each vial batch 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.

DISTRIBUTION OF THE PROFICIENCY TEST

The PT samples were distributed from the EURL on the 17th of March, 2025, and a replacement of PT samples to one NRL on the 31st of March, 2025. 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 temperature logger was included in each package to record the temperature every second hour during transport.

Twenty-eight NRLs received the PT within one day after the packages had been dispatched from the EURL, six NRLs within two days, and one NRL within three days (Table 3).

TABLE 3. Dates of arrival and start of analysis of proficiency test No. 39, 2025.

Arrival	Number of NRLs n=35	Start of analysis	Number of NRLs n=35
18th of March	27	18th of March	5
19th of March	6	19th of March	6
20th of March	1	20th of March	1
1st of April	1[a]	21st of March	4
		24th of March	10
		25th of March	4
		26th of March	2
		28th of March	1
		31st of March	1
		2nd of April	1[a]

[a] A new set of vials was sent to one NRL 31st of March, due to an issue in the laboratory. They arrived at 1st of April, and the analysis was started 2nd of April.

INSTRUCTIONS FOR LABORATORY PROCEDURES

The NRLs were recommended to follow ISO 10272-2 for performing PT 39. However, if their standard laboratory procedure followed a different method, they were allowed to use that method for the test.

The analysis was recommended to be started the same week as the PTs were dispatched from the EURL, and at the latest on the 31st 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 the week 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 summarised in Table 3.

Performance evaluation

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 two samples with homogeneous results (sample No. 2 and 10), 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 or triplicate vials were used in the PT (sample No. 1, 4 and 5, No. 2 and 10, and No. 7 and 8, respectively), the median and σMADe were calculated both for each single sample and for each group of samples prepared from the same batch of vials (both calculated values are presented in Table 5). The grouped values were used for the final performance evaluation, thus using the same scoring limits for all samples prepared from a specific vial batch.

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 yielded absolute z-scores values of ≤ 2.0 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 No. 39 was received by 35 NRLs and all of them reported the results of the analysis.

LABORATORY PROCEDURES

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. Seventeen laboratories started the analysis the same week the samples were dispatched from the EURL, 17 NRLs the week after, and one NRL two weeks after (Table 3).

Thirty-three NRLs reported to have followed the recommended method ISO 10272-2, either the method published 2017 (10), or ISO 10272-2:2017/Amd 1:2023 (22), or a combination of the two (1). Two NRLs used other methods: NMKL 119 3rd ed., 2007, and an internal method, respectively.

According to ISO 10272, *Campylobacter* spp. should be incubated in a microaerobic atmosphere, with oxygen content of 5% ± 2% and carbon dioxide 10% ± 3%. Of the 35 NRLs, 21 reported using commercial gas-generating kits, nine microaerobic incubators, and seven the Anoxomat® system to generate the appropriate microaerobic atmosphere. Two NRLs used more than one system.

ENUMERATION OF *CAMPYLOBACTER* SPP. (MANDATORY)

Of the 35 NRLs, 31 correctly reported *Campylobacter* spp. in all samples containing *Campylobacter* spp. and no detection of *Campylobacter* in the samples without *Campylobacter*. Six false negative results of *C. jejuni* samples, one of sample No. 2, two of sample No. 7, and three of sample No. 10, were reported by three NRLs. Three false positive results, two for sample No 3 (2.69 and 3.08 $\log_{10}$ cfu/g) and one for sample No. 6 (1.16 $\log_{10}$ cfu/g) were reported by two NRLs.

The median values (for all samples of the same batch in case of duplicate and triplicate vials) of the enumerations varied from 2.11 (sample No. 2 and 10) to 3.89 (sample No. 9) $\log_{10}$ cfu/g (Figure 1).

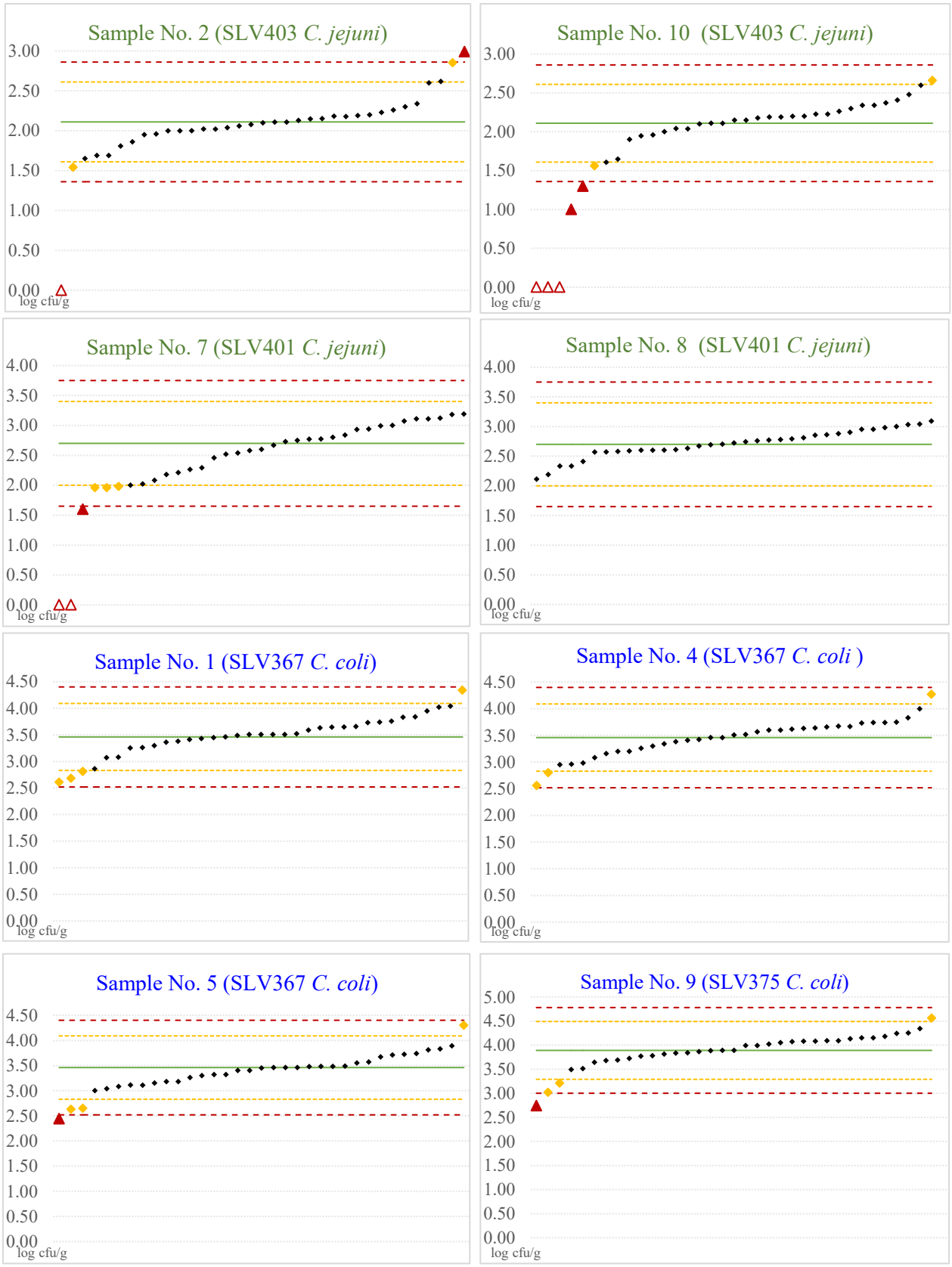

FIGURE 1. The quantity (log_{10} cfu/g) of *Campylobacter* spp. reported for each of the eight samples positive for *Campylobacter* by 35 NRLs in proficiency test No. 39, 2025. Samples reported as *Campylobacter* spp. not detected (< 1.00 log_{10} cfu/g) are shown as 0 in the figure and are represented by non-filled triangles. The median values (for all samples from the same batch in case of duplicate or triplicate 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 2.

According to the assessment, 29 NRLs (25 Member State NRLs, MS-NRLs) fulfilled the criterion for excellent or good performance and three NRLs (one MS-NRL) scored below the acceptable limit.

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

TABLE 4. Overall performance of 35 NRLs' enumeration of *Campylobacter* spp. in proficiency test No. 39, 2025.

		Number (proportion) of NRLs with performance within scores	
Grade	**Scoring limits for each performance grade**	Number (proportion) of NRLs All NRLs, n=35	Number (proportion) of NRLs MS-NRLs, n=28
Excellent	95.1–100%	21 (60%)	17 (61%)
Good	85.0–95.0%	8 (23%)	8 (29%)
Acceptable	70.0–84.9%	3 (9%)	2 (7%)
Needs improvement	57.0–69.9%	2 (6%)	1 (4%)
Poor	< 57.0%	1 (3%)	0 (0%)

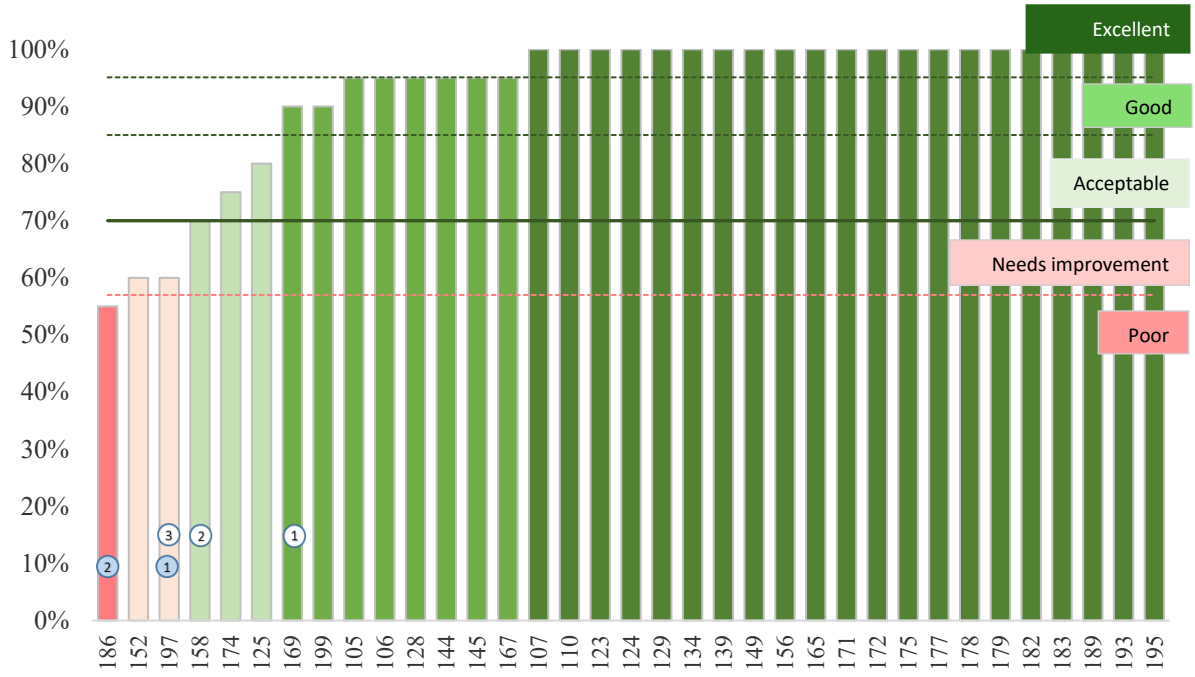

FIGURE 2. Distribution of the results of 35 participating NRLs, represented by lab ID, in combined score for enumerations of eight samples with *Campylobacter* and two samples without *Campylobacter* in proficiency test No. 39, 2025. Limits for grading of the overall performance are marked by horizontal lines. The numbers in white circles denote the number of negative results in samples containing *Campylobacter*, and the numbers in blue circles the number of false positive results.

TABLE 5. Results from the enumeration and z-scores of samples with *Campylobacter* in proficiency test No. 39, 2025. 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 4		Sample 5		Sample 7		Sample 8		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	4.02	1.80	2.60	1.96	3.74	0.90	3.81	1.12	3.19	1.42	3.03	0.96	4.24	1.18	2.66	2.20
106	3.38	−0.26	2.11	0.00	3.46	0.00	3.15	−1.00	1.96	−2.11	2.70	0.01	4.08	0.64	2.04	−0.28
107	3.83	1.19	2.13	0.08	3.83	1.19	3.89	1.38	3.00	0.88	2.33	−1.05	4.25	1.21	2.34	0.92
110	3.65	0.61	2.23	0.48	3.63	0.55	3.45	−0.03	3.11	1.19	2.88	0.53	4.34	1.52	2.23	0.48
123	3.43	−0.10	2.08	−0.12	3.20	−0.84	3.40	−0.19	2.60	−0.27	2.85	0.44	3.64	−0.84	2.11	0.00
124	3.51	0.16	2.02	−0.36	3.57	0.35	3.11	−1.12	2.29	−1.16	2.41	−0.82	3.81	−0.27	2.15	0.16
125	4.34	2.83	2.30	0.76	4.27	2.60	4.30	2.70	3.12	1.22	3.09	1.13	4.56	2.26	2.34	0.92
128	2.86	−1.93	1.86	−1.00	2.95	−1.64	2.63	−2.67	2.77	0.22	2.76	0.19	3.51	−1.28	2.19	0.32
129	3.30	−0.51	1.95	−0.64	3.16	−0.96	3.18	−0.90	2.21	−1.39	2.60	−0.27	4.13	0.81	2.30	0.76
134	3.59	0.42	2.18	0.28	3.41	−0.16	3.30	−0.51	2.84	0.42	2.79	0.27	4.02	0.44	2.11	0.00
139	3.25	−0.67	1.96	−0.60	3.60	0.45	3.04	−1.35	2.52	−0.50	2.58	−0.33	3.84	−0.17	2.18	0.28
144	3.26	−0.64	2.20	0.36	3.46	0.00	3.40	−0.19	2.00	−1.99	2.74	0.13	3.77	−0.40	1.56	−2.20
145	3.36	−0.32	2.06	−0.20	3.60	0.45	3.57	0.35	1.98	−2.05	2.72	0.07	3.49	−1.35	2.00	−0.44
149	3.49	0.10	2.04	−0.28	3.62	0.51	3.46	0.00	2.58	−0.33	2.57	−0.36	3.89	0.00	1.90	−0.84
152	2.68	−2.51	1.54	−2.28	2.80	−2.12	3.32	−0.45	1.60	−3.14	2.19	−1.45	3.21	−2.29	1.30	−3.24
156	3.07	−1.25	2.26	0.60	3.38	−0.26	3.48	0.06	2.77	0.22	2.33	−1.05	3.88	−0.03	2.04	−0.28
158	3.66	0.64	2.99	3.52	3.66	0.64	3.18	−0.90	<1.00	−4.86[a]	2.95	0.73	3.83	−0.20	<1.00	−4.44[a]
165	3.95	1.57	2.10	−0.04	3.74	0.90	3.83	1.19	2.99	0.85	3.04	0.99	3.99	0.34	2.26	0.60
167	3.41	−0.16	2.85	2.96	3.42	−0.13	3.46	0.00	2.54	−0.44	2.63	−0.19	3.69	−0.67	2.37	1.04
169	3.84	1.22	1.69	−1.68	3.26	−0.64	3.74	0.90	2.75	0.16	2.60	−0.27	4.07	0.61	<1.00	−4.44[a]
171	3.45	−0.03	2.02	−0.36	3.73	0.87	3.55	0.29	2.93	0.67	2.69	−0.01	3.89	0.00	1.61	−2.00
172	3.51	0.16	2.15	0.16	3.30	−0.51	3.48	0.06	2.08	−1.77	2.60	−0.27	4.05	0.54	2.48	1.48
174	2.81	−2.09	2.11	0.00	2.96	−1.61	2.65	−2.60	1.96	−2.11	2.11	−1.68	2.74	−3.88	2.60	1.96
175	3.74	0.90	2.19	0.32	3.64	0.58	3.72	0.84	3.07	1.08	2.90	0.59	4.08	0.64	2.20	0.36
177	3.76	0.96	2.15	0.16	3.67	0.67	3.48	0.06	2.94	0.70	2.95	0.73	4.15	0.88	2.23	0.48
178	3.63	0.55	1.81	−1.20	3.52	0.19	3.49	0.10	2.73	0.10	2.67	−0.07	3.99	0.34	2.15	0.16
179	3.52	0.19	2.00	−0.44	3.67	0.67	3.67	0.67	2.46	−0.67	2.77	0.22	4.09	0.67	2.10	−0.04
182	3.08	−1.22	2.00	−0.44	2.98	−1.54	3.00	−1.48	2.18	−1.48	2.59	−0.30	4.15	0.88	2.20	0.36
183	3.51	0.16	2.00	−0.44	3.08	−1.22	3.08	−1.22	3.18	1.39	2.78	0.24	3.68	−0.71	1.95	−0.64
186	2.61	−2.73	1.69	−1.68	2.56	−2.89	2.44	−3.28	2.02	−1.94	2.57	−0.36	3.02	−2.93	1.96	−0.60
189	3.73	0.87	2.18	0.28	3.75	0.93	3.71	0.80	2.80	0.30	3.00	0.88	4.09	0.67	2.19	0.32
193	3.46	0.00	2.62	2.04[b]	3.51	0.16	3.46	0.00	2.67	−0.07	2.86	0.47	3.78	−0.37	2.41	1.20
195	3.64	0.58	1.65	−1.84	3.34	−0.39	3.26	−0.64	2.26	−1.25	2.61	−0.24	3.86	−0.10	1.65	−1.84
197	3.51	0.16	<1.00	−4.44[a]	3.20	−0.84	3.11	−1.12	<1.00	−4.86[a]	2.81	0.33	3.72	−0.57	<1.00	−4.44[a]
199	4.04	1.86	2.34	0.92	4.00	1.73	3.32	−0.45	3.11	1.19	2.98	0.82	4.18	0.98	1.00	−4.44
Median[c]	**3.46**	3.51	**2.11**	2.10	**3.46**	3.51	**3.46**	3.45	**2.70**	2.60	**2.70**	2.72	**3.89**	3.89	**2.11**	2.15
MADe	**0.21**	0.21	**0.15**	0.10	**0.21**	0.21	**0.21**	0.26	**0.24**	0.39	**0.24**	0.14	**0.20**	0.20	**0.15**	0.19
σMADe	**0.31**	0.31	**0.25[d]**	0.25[d]	**0.31**	0.31	**0.31**	0.39	**0.35**	0.58	**0.35**	0.25[d]	**0.30**	0.30	**0.25[d]**	0.28
±2σMADe	4.09	2.83	2.61	1.61	4.09	2.83	4.09	2.83	3.40	1.99	3.40	1.99	4.49	3.29	2.61	1.61
±3σMADe	4.40	2.52	2.86	1.36	4.40	2.52	4.40	2.52	3.75	1.64	3.75	1.64	4.78	3.00	2.86	1.36

[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 all samples of duplicate/triplicate vials 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 two rows below).

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

SPECIES IDENTIFICATION OF *CAMPYLOBACTER* SPP. (VOLUNTARY)

Thirty (86%) of the 35 NRLs reported results of species identification. Twenty-eight of the 30 NRLs reported correct species in all eight samples that had been inoculated with *Campylobacter* spp. Two NRLs reported one and three misidentifications, respectively, and the same NRLs reported two and one false negative results, respectively, which could not be species identified (Table 6 and Figure 3).

The isolated *Campylobacter* spp. were identified by biochemical tests and/or molecular methods: matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) or polymerase chain reaction (PCR). The biochemical tests included detection of catalase, hippurate hydrolysis and indoxyl acetate hydrolysis.

Twenty-three of the 35 NRLs reported that they used MALDI-TOF MS for the species identification, in nine cases combined with other techniques. Ten NRLs used one or more PCR assays, in five cases combined with other techniques. Four NRLs reported to have used one of the PCR assays described in ISO 10272-2:2017/Amd 1:2023, one to have used the PCR assay of Best et al. (2003), and two to have used the PCR assay of Denis et al. (1999). Six NRLs used biochemical tests, in four cases combined with MALDI-TOF MS.

Twenty-one 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 6. Species identification reported by 30 NRLs in the voluntary part of proficiency test No. 39, 2025. Incorrect results are in bold text: red for false negative results, blue for false positive results, and orange for misidentifications.

		No. of NRLs reporting					
Sample No.	**Species**	*Campylobacter jejuni*	*Campylobacter coli*	*Campylobacter lari*	*Campylobacter* but unable to identify species	No growth at all	Growth of other, not *Campylobacter*
1	*Campylobacter coli*		30				
2	*Campylobacter jejuni*	29	**1**				
3	*Escherichia coli*				**1**	5	24
4	*Campylobacter coli*		29	**1**			
5	*Campylobacter coli*		29	**1**			
6	Negative				**1**	16	13
7	*Campylobacter jejuni*	29					**1**
8	*Campylobacter jejuni*	30					
9	*Campylobacter coli*		29	**1**			
10	*Campylobacter jejuni*	28					**2**

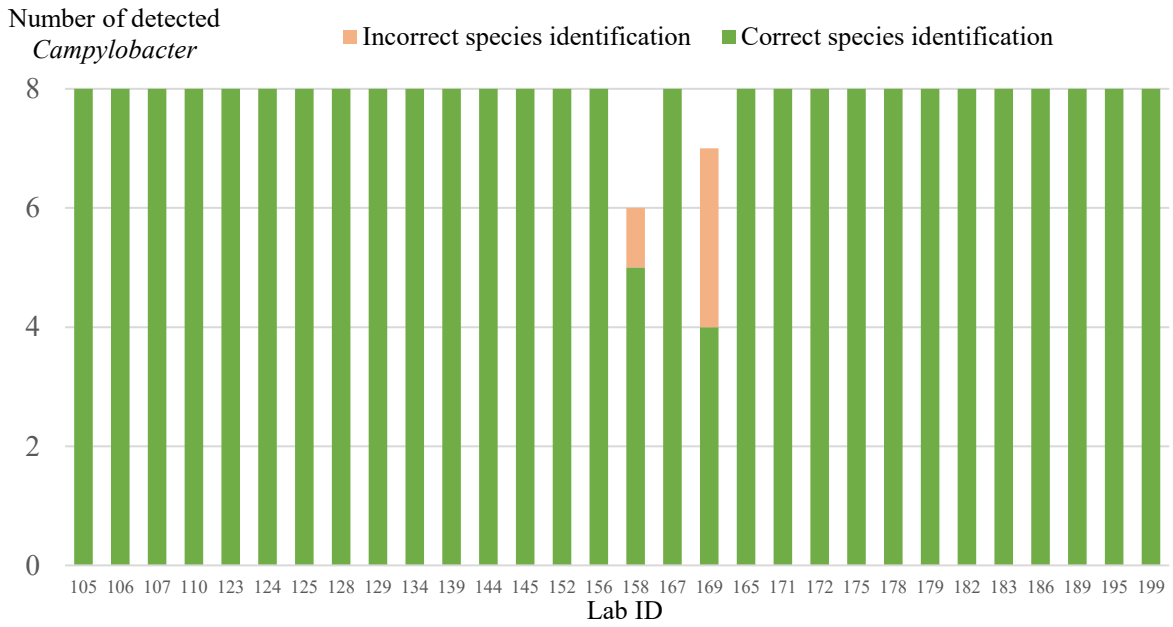

FIGURE 3. Results by 30 NRLs reporting results for species identification in the voluntary part of proficiency test No. 39, 2025.

PERFORMANCE IN IDENTIFICATION OF *CAMPYLOBACTER* SPP.

Twenty-eight (22 MS-NRLs) of the 30 NRLs reporting results for species identification of *Campylobacter* fulfilled the criterion for excellent performance in identification of *Campylobacter* spp., and one MS-NRL scored below the acceptable limit (Table 7). The overall median sensitivity in correctly identifying *Campylobacter* spp. was 100% (50% central range: 100%–100%).

TABLE 7. Overall performance of 30 NRLs ' sensitivity in correctly identifying *Campylobacter* spp. in the voluntary part of proficiency test No. 39, 2025.

		Performance in identification of *Campylobacter* spp.	
Grade	**Sensitivity**	Number (proportion) of NRLs All NRLs, n=30	Number (proportion) of NRLs MS-NRLs, n=24
Excellent	95.1–100%	28 (93%)	22 (92%)
Good	85.0–95.0%	0 (0%)	0 (0%)
Acceptable	70.0–84.9%	1 (3%)	1 (4%)
Needs improvement	57.0–69.9%	1 (3%)	1 (4%)
Poor	< 57.0%	0 (0%)	0 (0%)

References

ISO 33405:2024: Reference materials — Approaches for characterization and assessment of homogeneity and stability. International Organization for Standardization.

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.

ISO 10272-2:2017/Amd 1:2023: Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of *Campylobacter* spp. – Part 2: Colony-count technique. Amendment 1: Inclusion of methods for molecular confirmation and identification of thermotolerant *Campylobacter* spp. and changes in the performance testing of culture media. International Organization for Standardization.

ISO 22117:2019: Microbiology of food and animal feeding stuffs – Specific requirements and guidance for proficiency testing by interlaboratory comparison. International Organization for Standardization.

NMKL 119, 3rd ed. 2007: Thermotolerant *Campylobacter*. Detection, semi-quantitative and quantitative determination in foods and drinking water. Nordic Committee on Food Analysis.

Best EL, Powell EJ, Swift C, Grant KA, Frost JA. Applicability of a rapid duplex real-time PCR assay for speciation of *Campylobacter jejuni* and *Campylobacter coli* directly from culture plates. FEMS Microbiology Letters. 2003;229(2):237–41. doi: 10.1016/S0378-1097(03)00845-0

Denis M, Soumet C, Rivoal K, Ermel G, Blivet D, Salvat G, Colin, P. Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. Letters in Applied Microbiology. 1999;29(6):406–10. doi: 10.1046/j.1472-765x.1999.00658.x

The Swedish Veterinary Agency (SVA) is an expert authority with contingency missions. SVA promotes animal and human health, Swedish animal husbandry and our environment through diagnostics, research, preparedness, and advice. The authority is under the Ministry of Rural Affairs and Infrastructure.

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