

# **EURL-Campylobacter Proficiency Test Report**

## **PT 40. Detection and species identification of Campylobacter**



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**EURL-Campylobacter Proficiency Test Report**

**PT 40. Detection and species identification of *Campylobacter***

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**Cover image** *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 40 on detection and species identification of *Campylobacter* spp. in chicken caecal content in March 2025. The PT included detection of *Campylobacter* spp. in 18 samples of chicken caecal material mixed with vials with or without freeze-dried *Campylobacter*. The objective was to assess the performance of the national reference laboratories (NRLs) in detection and species identification of *Campylobacter* in chicken caecal content.

As a voluntary (educational) part of the test, two additional vials with freeze-dried *Campylobacter*, along with chicken caecal material, were sent to participating NRLs.

Participation in PT 40 was mandatory for at least one NRL per Member State (MS). Thirty-four NRLs in 27 EU MSs (some MSs have more than one NRL) and in four non-EU countries received the PT and responses were reported from all of them. Thirty-two NRLs reported to have followed the recommended method of ISO 10272-1, and two NRLs used other methods.

A combined five-level grading scale for performance in detection was based on minimum limits for sensitivity in detection of low-level samples, sensitivity in detection of high-level samples and specificity. Twenty-nine NRLs (85%) fulfilled the criterion for excellent or good performance and three NRLs (all Member State NRLs, MS-NRLs) scored below the acceptable limit.

Thirty-three NRLs (97%) fulfilled the criterion for excellent or good performance in identification of *Campylobacter* spp., and none scored below the acceptable limit.

In summary, the majority of the NRLs met the criteria for excellent or good performance in both detection and species identification. Three MS-NRLs scored below the acceptable limit in detection.

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# Abbreviations

|              |  |
|--------------|--|
| BPW          | Buffered peptone water   |
| <i>C.</i>    | <i>Campylobacter</i>   |
| 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)  |
| 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  |

# Introduction

Proficiency test (PT) No. 40 on detection and species identification of *Campylobacter* spp. in chicken caecal content was organised by the EU reference laboratory (EURL) for *Campylobacter* in March 2025. Thirty-four national reference laboratories (NRLs) in 27 EU Member States (MSs, some MSs have more than one NRL) and in four non-EU countries received the PT. All 34 NRLs reported the test results and operational details to the EURL.

Thirty-one NRLs reported that they were accredited for detection of *Campylobacter* and 23 that they were accredited for enumeration of *Campylobacter*. Eight NRLs were accredited for detection only, and three NRLs were accredited neither for detection nor enumeration of *Campylobacter* spp.

The PT included detection and identification of *Campylobacter* spp. in 18 core samples, and as a voluntary addition two educational samples, of chicken caecal material mixed with vials with or without freeze-dried *Campylobacter* (Table 1). The objective was to assess the performance of the NRLs in detection and species identification of *Campylobacter* in chicken caecal content.

**TABLE 1.** Contents of the 18 vials for core samples No. 11-28 and two vials for educational samples No. 29-30 distributed to the NRLs in proficiency test No. 40, 2025.

| Sample No. | Species   | Level <i>Campylobacter</i> <sup>b</sup><br>( $\log_{10}$ cfu/vial) | Level <i>E. coli</i> <sup>c</sup><br>( $\log_{10}$ cfu/vial) | Standard deviation <sup>d</sup><br>( $\log_{10}$ cfu) | Batch No. |
|------------|---|--|--|---|-----------|
| 11         | <i>Escherichia coli</i>   |  | 5.81   | 0.05  | SVA096    |
| 12         | <i>Campylobacter coli</i>                                       | 5.52 high  |  | 0.05  | SVA083    |
| 13         | <i>Campylobacter lari</i>                                       | 4.43 low   |  | 0.06  | SVA087    |
| 14         | <i>Campylobacter jejuni</i> <sup>a</sup>                        | 6.43 high  |  | 0.05  | SVA090    |
| 15         | <i>Escherichia coli</i>   |  | 5.81   | 0.05  | SVA096    |
| 16         | <i>Campylobacter jejuni</i> <sup>a</sup>                        | 4.00 low   |  | 0.06  | SVA085    |
| 17         | Negative  |  |  |   |           |
| 18         | <i>Campylobacter jejuni</i> <sup>a</sup>                        | 4.00 low   |  | 0.06  | SVA085    |
| 19         | <i>Campylobacter coli</i>                                       | 5.52 high  |  | 0.05  | SVA083    |
| 20         | Negative  |  |  |   |           |
| 21         | <i>Campylobacter jejuni</i> <sup>a</sup>                        | 6.43 high  |  | 0.05  | SVA090    |
| 22         | Negative  |  |  |   |           |
| 23         | <i>Campylobacter jejuni</i> <sup>a</sup>                        | 4.00 low   |  | 0.06  | SVA085    |
| 24         | <i>Campylobacter coli</i>                                       | 3.96 low   |  | 0.05  | SVA089    |
| 25         | <i>Escherichia coli</i>   |  | 5.81   | 0.05  | SVA096    |
| 26         | <i>Campylobacter coli</i>                                       | 5.01 high  |  | 0.08  | SVA081    |
| 27         | <i>Campylobacter lari</i>                                       | 4.43 low   |  | 0.06  | SVA087    |
| 28         | <i>Campylobacter coli</i>                                       | 5.01 high  |  | 0.08  | SVA081    |
| 29         | <i>Campylobacter lari</i> &<br><i>Campylobacter upsaliensis</i> | 5.50   |  | 0.06  | SVA099    |
| 30         | <i>Campylobacter jejuni</i> &<br><i>Campylobacter lari</i>      | 4.48   |  | 0.12  | SVA015    |

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

<sup>b</sup>Total quantity of *Campylobacter* in each vial.

<sup>c</sup>Total quantity of *Escherichia coli* in each vial.

<sup>d</sup>According to homogeneity test of ten vials after the production. The maximum standard deviation allowed was 0.15  $\log_{10}$  cfu.

The educational samples were prepared to mimic caecal samples from chickens at a farm with ecological or free-range rearing, i.e. birds kept outdoors. This means that other *Campylobacter* species than those most commonly found in chickens could be present, and that there could be more than one *Campylobacter* strain in the same sample. The strains were however chosen to be possible to isolate using the recommended method, i.e., they were able to grow on modified charcoal cefoperazone deoxycholate (mCCD) agar at 41.5 °C.

## 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*.
- Detection of *Campylobacter* spp.: Determination of the presence or absence of *Campylobacter* spp.
- 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 CAECAL MATERIAL

The chicken caeca 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 (2.7% during 2023).

On arrival, the chicken caecal material was tested in triplicate for detection by direct streak on mCCD agar. The chicken caeca tested negative for detection of *Campylobacter* by direct streak, but a moderate background flora was present. The chicken caeca were packed in zip bags and freeze-stored at -20 °C until distribution of the PT. In addition, chicken skin samples from the same flock tested negative for *Campylobacter* by direct streak of rinse with buffered peptone water (BPW) on mCCD agar and three months later, after freeze-storage at -20 °C, also by enrichment with Bolton and Preston broth.

Three days prior to distribution of the PT, the caecal material was thawed and homogenised with BPW by stomaching. Six ml of caecal suspension were thereafter aliquoted into plastic tubes. The plastic tubes were stored at 5 °C until distribution of the PT. Each sample was intended to mimic a sample pooled from up to 30 chicken caeca, according to the procedure for pooling contents from whole poultry caeca described in ISO 6887-6.

## PRODUCTION AND QUALITY CONTROL OF THE VIALS

The vials with freeze-dried bacterial cultures used in the PT were produced by the EURL and tested for stability and homogeneity. The standard deviation from the homogeneity testing of ten vials analysed in repeatable conditions is included in Table 1.

To ensure that the level of instability during transport conditions did not affect the performance evaluation, each combination of vial batch and matrix was prepared and tested under various transport conditions (Table 2). The tests were performed according to procedure C (direct streak) in ISO 10272-1:2017 (including ISO 10272-1:2017/Amd 1:2023), as the only procedure or combined with detection procedure A (enrichment in Bolton broth) and procedure B (enrichment in Preston broth), on three occasions. The plating was done on mCCD and Butzler agar.

The stability 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 one week after dispatch (“worst case” conditions), after the last date for start of analysis by the participants, on vials stored in “worst case” conditions (styrofoam box stored at room temperature for 48 h) before storage at -20 °C until start of analysis. In addition, the stability of vials with *Campylobacter* was checked by performing viable count on blood agar.

**TABLE 2.** Outline and results of stability testing under transport conditions for proficiency test No. 40, 2025.

| Test occasion           | Storage condition <sup>a</sup> | Test method <sup>b</sup> | Number of samples tested                      | Result <sup>c</sup> |
|-------------------------|--------------------------------|--------------------------|---|---------------------|
| Before dispatch         | Best case                      | C                        | Each vial batch with <i>Campylobacter</i> × 2 | +                   |
| Before dispatch         | Worst case                     | A + B + C + VC           | Each vial batch with <i>Campylobacter</i> × 2 | +                   |
| One week after dispatch | Worst case                     | C + VC                   | Each vial batch with <i>Campylobacter</i> × 3 | +                   |

<sup>a</sup> 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.

<sup>b</sup> Detection procedure according to ISO 10272-1:2017 and ISO 10272-1:2017/Amd 1:2023: direct plating (C) on mCDD and Butzler agar or after enrichment in Bolton (A) or Preston broth (B). The detection procedures were applied in samples of chicken caecal material mixed with vials. VC: viable count on blood agar plates of vials with *Campylobacter*.

<sup>c</sup> A plus indicates that *Campylobacter* could be detected in all samples with *Campylobacter* after indicated storage condition.

## DISTRIBUTION OF THE PROFICIENCY TEST

The PT samples were distributed from the EURL on the 17<sup>th</sup> of March, 2025, and a replacement of PT samples to one NRL on the 31<sup>st</sup> 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 18 numbered vials, each containing freeze-dried material with or without *Campylobacter* spp., and 18 plastic tubes with 6 ml of chicken caecal material, one tube for each of the 18 vials. Each participant in the voluntary part of the PT received two additional vials and plastic tubes with 6 ml of chicken caecal material, which served as educational samples. A temperature logger was included in each package to record the temperature every second hour during transport.

Twenty-six NRLs received the PT within one day after the packages had been dispatched from the EURL, seven 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. 40, 2025.

| Arrival                               | Number of NRLs<br>n=34 |  | Start of analysis                     | Number of NRLs<br>n=34 |
|---------------------------------------|------------------------|--|---------------------------------------|------------------------|
|                                       |                        |  |                                       |                        |
| 18 <sup>th</sup> of March             | 25                     |  | 18 <sup>th</sup> of March             | 12                     |
| 19 <sup>th</sup> of March             | 7                      |  | 19 <sup>th</sup> of March             | 15                     |
| 20 <sup>th</sup> of March             | 1                      |  | 20 <sup>th</sup> of March             | 2                      |
| 1 <sup>st</sup> of April <sup>a</sup> | 1                      |  | 21 <sup>st</sup> of March             | 3                      |
|                                       |                        |  | 24 <sup>th</sup> of March             | 1                      |
|                                       |                        |  | 3 <sup>rd</sup> of April <sup>a</sup> | 1                      |

<sup>a</sup> A new set of vials and matrix (frozen) was sent to one NRL 31<sup>st</sup> of March, due to an issue in the laboratory. They arrived at 1<sup>st</sup> of April, and the analysis was started 3<sup>rd</sup> of April.

## INSTRUCTIONS FOR LABORATORY PROCEDURES

The NRLs were recommended to follow ISO 10272-1:2017, procedure C, direct plating for performing PT 40. However, if their standard laboratory procedure followed a different method, they were allowed to use that method for the test.

The PT analyses were recommended to be started as soon as possible after the arrival and at the latest on the 21<sup>st</sup> of March. Instructions for preparation of the samples from the vials and matrix were included in the packages and were also sent out by e-mail the week before the PT distribution. Until start of analysis, chicken caecal material and vials were recommended to be stored at cold temperature (between 1 °C and 8 °C). If the analysis was not started the same day as arrival, the vials were recommended to be stored at –20 °C or –70 °C. The dates for start of analysis are summarised in Table 3.

# Performance evaluation

The NRLs' performance in sensitivity in detection (of *Campylobacter*-positive samples in total and in low-level and high-level samples separately), sensitivity in identification, and accuracy in detection of *Campylobacter* in samples with and without *Campylobacter* were calculated from the final results as reported by each participant.

The sensitivity was calculated based on the NRLs ability to correctly detect *Campylobacter* spp. and identify *Campylobacter* species in the samples containing *Campylobacter*. Correct detection of all *Campylobacter*-positive samples (in the low-level and high-level category, respectively), resulted in a sensitivity in detection of 100%. Correct identification of all *Campylobacter* species in positive samples in which *Campylobacter* spp. were detected resulted in a sensitivity in species identification of 100%.

For determining the performance in detection of negative *Campylobacter* samples, the specificity was calculated for each NRL. Correct identification of all samples without *Campylobacter* as non-*Campylobacter* samples resulted in a specificity of 100%.

The accuracy was also calculated, giving an overall performance of the results of correct detection of *Campylobacter* spp. in samples with *Campylobacter* and correct identification of samples without *Campylobacter* as non-*Campylobacter* samples. The accuracy was calculated as total number of correct detection results divided by total number of samples.

A combined five-level grading scale for performance in detection was based on the number of correct results of detection for the three categories of samples (low-level *Campylobacter*-positive samples, high-level *Campylobacter*-positive samples, and *Campylobacter*-negative samples) according to Table 4. The scoring limits for the low- and high-level categories were based on the total detection rates of low-level and high-level samples (93.6% and 100.0%, respectively). The cut-off for good performance in species identification of *Campylobacter* was set to 85.0%.

**TABLE 4.** The minimum number of correct results (*Campylobacter* detected or not detected) needed for each combined performance grade, and the corresponding measures of sensitivity (Se), specificity (Sp), and accuracy (Acc), in proficiency test No. 40, 2025. Performance scoring below any of the limits for the performance grade Needs improvement was graded as Poor.

| Performance grade        | Category of samples |                     |                   | Measures on the lower limit of each grade (%) |         |          |       |       |
|--------------------------|---------------------|---------------------|-------------------|---|---------|----------|-------|-------|
|                          | Low-level<br>(n=6)  | High-level<br>(n=6) | Negative<br>(n=6) | Se low  | Se high | Se total | Sp    | Acc   |
| <b>Excellent</b>         | 6                   | 6                   | 6                 | 100.0   | 100.0   | 100.0    | 100.0 | 100.0 |
| <b>Good</b>              | 5                   | 6                   | 6                 | 83.3  | 100.0   | 91.7     | 100.0 | 94.4  |
| <b>Acceptable</b>        | 4                   | 5                   | 5                 | 66.7  | 83.3    | 75.0     | 83.3  | 77.8  |
| <b>Needs improvement</b> | 3                   | 4                   | 4                 | 50.0  | 66.7    | 58.3     | 66.7  | 61.1  |

# Results

Proficiency test No. 40 was received by 34 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 four days after dispatch. Thirty-three laboratories started the analysis the same week the samples were dispatched from the EURL, and one NRL the week after (Table 3).

Thirty-two NRLs reported to have followed the recommended method ISO 10272-1, either the method published 2017 (11), or ISO 10272-1:2017/Amd 1:2023 (21). Two NRLs used other methods.

According to ISO 10272, *Campylobacter* spp. should be incubated in a microaerobic atmosphere, with oxygen content of  $5\% \pm 2\%$  and carbon dioxide  $10\% \pm 3\%$ . Of the 34 NRLs, 18 reported using commercial gas-generating kits, eight microaerobic incubators, and nine the Anoxomat® system to generate the appropriate microaerobic atmosphere. Two NRLs used more than one system.

All except two NRLs performed direct plating on selective plates as recommended, in 28 cases as the only procedure and in four cases in addition to enrichment procedures. Two NRLs performed enrichment in Bolton broth (in addition to direct plating) and four in Preston broth (two in addition to direct plating and two as the only procedure). Another additional procedure used for detection was digital droplet polymerase chain reaction (ddPCR). Twenty-nine NRLs used one procedure only and five NRLs two procedures for the detection part.

All 34 NRLs used mCCD agar and 22 of them plated on at least one additional medium. Other media used for plating were Butzler agar (16), CampyFood® agar (2), Karmali agar (1), Preston agar (1), Skirrow agar (1), RAPID' *Campylobacter* agar (1), CASA® agar (1), CHROMagar™ *Campylobacter* (1), CAT agar (1) and mCCD agar with addition of 1.5 mg/l tazobactam (1).

The presumptive *Campylobacter* colonies were confirmed by typical microscopic morphology and motility, positive oxidase test, lack of aerobic growth at 25 °C and/or molecular methods: matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) or polymerase chain reaction (PCR). Eighteen of the 34 NRLs used microscopic examination as part of the confirmation procedure. Sixteen NRLs used oxidase test, in 13 cases in combination with aerobic growth at 25 °C. Twenty-three NRLs used MALDI-TOF MS for confirmation, in ten cases combined with additional techniques other than microscopic examination. Twelve NRLs used one or more PCR assays, in ten cases combined with additional techniques other than microscopy. Four NRLs reported to have used PCR assays described in ISO 10272-1:2017/Amd 1:2023 (in one case combined with the PCR assay of Maher et al. 2003), two to have used the PCR assay of Best et al. (2003), and two to have used the PCR assay of Denis et al. (1999).

The isolated *Campylobacter* spp. were identified by biochemical tests and/or molecular methods: MALDI-TOF MS or PCR. The biochemical tests included detection of catalase, hippurate hydrolysis and indoxyl acetate hydrolysis.

Nineteen NRLs used MALDI-TOF MS for species identification, in six cases combined with other techniques. Ten NRLs used one or more PCR assays for the species identification, in seven cases combined with other techniques. Five NRLs reported to have used PCR assays described in ISO 10272-1:2017/Amd 1:2023 (in one case combined with the PCR assay of Maher et al. 2003), two to have used the PCR assay of Best et al. (2003) and two to have used the PCR assay of Denis et al. (1999). Nine NRLs used biochemicals tests (at least detection of catalase), in six cases combined with MALDI-TOF MS and/or PCR.

Nineteen NRLs used one technique only (a set of biochemical tests and/or tests of growth regarded as one technique), seven NRLs combined two techniques, and one NRL three techniques.

## DETECTION OF CAMPYLOBACTER

Of the 34 NRLs, 28 reported correct results of detection, i.e. detection of *Campylobacter* in all twelve samples containing *Campylobacter* spp. and no detection of *Campylobacter* in the six samples without *Campylobacter* (Figure 1). The same 28 NRLs also reported correct species in all samples containing *Campylobacter* spp.

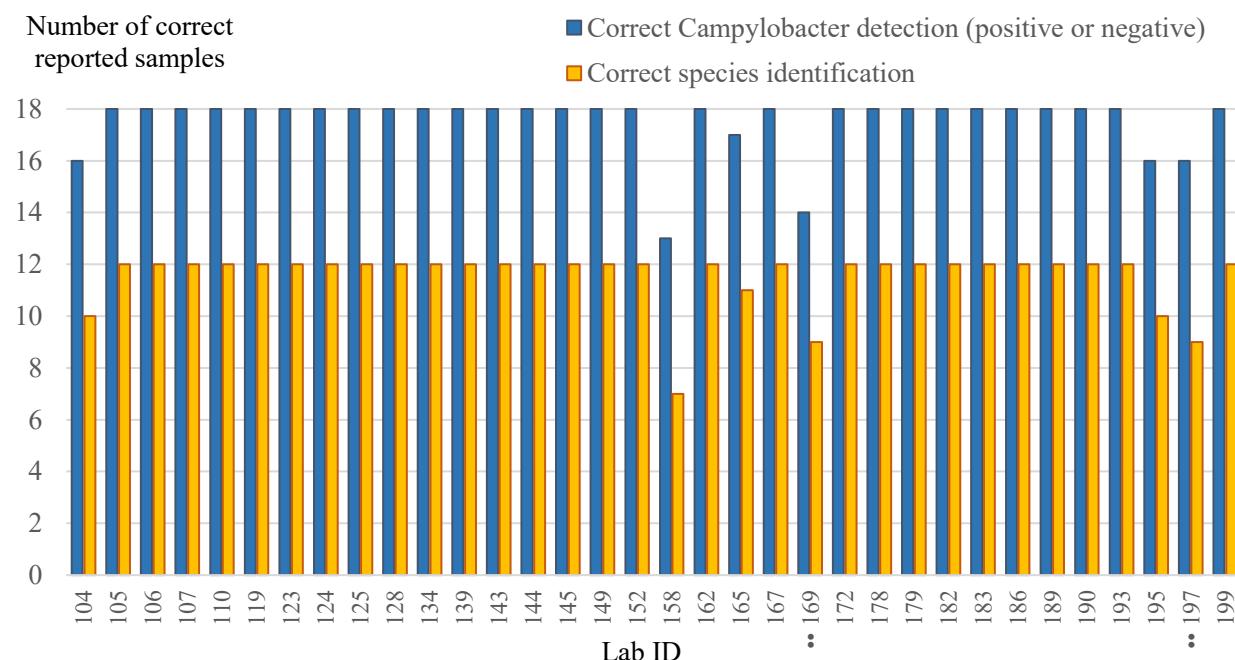
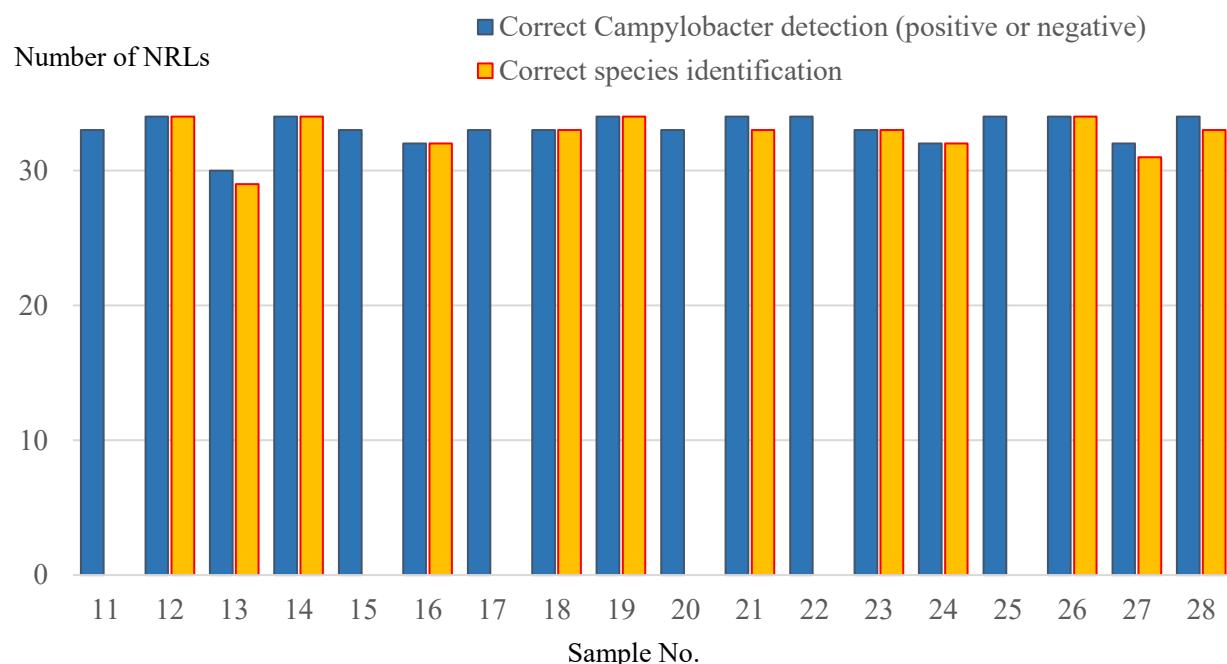


FIGURE 1. Distribution of correct results by 34 NRLs participating in proficiency test No. 40, 2025, in the detection and species identification of *Campylobacter* spp. in chicken caecal content. A dot • denotes a false positive result.

All 34 NRLs reported correct results of detection for six of the twelve *Campylobacter*-negative samples and all high-level samples, whereas twelve false negative results were reported for the six low-level samples (Figure 2, Table 5). The number of false negative results reported for samples containing *Campylobacter* varied from zero to four. The highest number of false negative results were reported for the vial batch SVA087 containing *C. lari* (sample No. 13 and 27), with an average number of 3.0 false negative results per sample. The other two low-level vial batches SVA089 containing *C. coli* (sample No. 24) and SVA085 containing *C. jejuni* (sample No. 16, 18, and 23) had an average number of 2.0 and 1.3 false negative results per sample, respectively.



**FIGURE 2.** Number of the 34 NRLs participating in proficiency test No. 40, 2023, that correctly reported results in the detection and species identification of *Campylobacter* in 18 chicken caecal content samples.

## SPECIES IDENTIFICATION OF CAMPYLOBACTER

Thirty-two NRLs reported correct species in all samples where *Campylobacter* spp. had been detected, whereas four misidentifications, one each of sample No. 13, 21, 27 and 28, were reported (Figure 2, Table 5). Two NRLs reported one and three misidentifications, respectively (Figure 1). These two NRLs also reported two false positive results each. One NRL reported two *C. lari* and one *C. jejuni* as *C. coli*, and the same species identification was given the two false positive results.

Both NRLs reporting false positive results and misidentifications reported to have used PCR for confirmation (one NRL in combination with biochemical tests) and species identification.

**TABLE 5.** Results of detection and species identification in 18 samples of chicken caecal content in proficiency test No. 40, 2025. In total 34 NRLs performed species identification. Incorrect results are in bold text: red for false negative results, blue for false positive results, and orange for misidentifications.

| Sample<br>No. Species                 | Campylobacter jejuni | Campylobacter coli | Campylobacter lari | Campylobacter spp. but unable to identify species | Growth of other, not Campylobacter | No growth at all | Number of NRLs reporting |
|---------------------------------------|----------------------|--------------------|--------------------|---|------------------------------------|------------------|--------------------------|
| <b>11</b> <i>Escherichia coli</i>     | <b>1</b>             |                    |                    |   | 31                                 | 2                |                          |
| <b>12</b> <i>Campylobacter coli</i>   |                      | 34                 |                    |   |                                    |                  |                          |
| <b>13</b> <i>Campylobacter lari</i>   | <b>1</b>             |                    | 29                 |   | <b>2</b>                           | <b>2</b>         |                          |
| <b>14</b> <i>Campylobacter jejuni</i> | 34                   |                    |                    |   |                                    |                  |                          |
| <b>15</b> <i>Escherichia coli</i>     | <b>1</b>             |                    |                    |   | 31                                 | 2                |                          |
| <b>16</b> <i>Campylobacter jejuni</i> | 32                   |                    |                    |   | <b>1</b>                           | <b>1</b>         |                          |
| <b>17</b> –                           |                      |                    |                    | <b>1</b>  | 13                                 | 20               |                          |
| <b>18</b> <i>Campylobacter jejuni</i> | 33                   |                    |                    |   | <b>1</b>                           |                  |                          |
| <b>19</b> <i>Campylobacter coli</i>   |                      | 34                 |                    |   |                                    |                  |                          |
| <b>20</b> –                           |                      |                    |                    | <b>1</b>  | 13                                 | 20               |                          |
| <b>21</b> <i>Campylobacter jejuni</i> | 33                   | <b>1</b>           |                    |   |                                    |                  |                          |
| <b>22</b> –                           |                      |                    |                    |   | 12                                 | 22               |                          |
| <b>23</b> <i>Campylobacter jejuni</i> | 33                   |                    |                    |   | <b>1</b>                           |                  |                          |
| <b>24</b> <i>Campylobacter coli</i>   |                      | 32                 |                    |   | <b>2</b>                           |                  |                          |
| <b>25</b> <i>Escherichia coli</i>     |                      |                    |                    |   | 31                                 | 3                |                          |
| <b>26</b> <i>Campylobacter coli</i>   | 34                   |                    |                    |   |                                    |                  |                          |
| <b>27</b> <i>Campylobacter lari</i>   | <b>1</b>             |                    | 31                 |   | <b>1</b>                           | <b>1</b>         |                          |
| <b>28</b> <i>Campylobacter coli</i>   | 33                   | <b>1</b>           |                    |   |                                    |                  |                          |

## PERFORMANCE IN DETECTION AND SPECIES IDENTIFICATION OF CAMPYLOBACTER spp.

The results of using the five-level grading scale for the overall assessment of the NRLs' detection of *Campylobacter* spp. are presented in Table 6. According to the assessment, 29 NRLs (24 Member State NRLs, MS-NRLs) fulfilled the criterion for excellent or good performance and three NRLs (all MS-NRLs) scored below the acceptable limit.

**TABLE 6.** Combined performance grades in detection of *Campylobacter* spp. in proficiency test No. 40, 2025.

| <b>Grade</b>             | <b>Combined performance in detection of <i>Campylobacter</i> spp.</b> |  |
|--------------------------|---|--|
|                          | Number (proportion) of NRLs<br>All NRLs, n=34                         | Number (proportion) of NRLs<br>MS-NRLs, n=28 |
| <b>Excellent</b>         | 28 (82%)  | 23 (82%)                                     |
| <b>Good</b>              | 1 (3%)  | 1 (4%)                                       |
| <b>Acceptable</b>        | 2 (6%)  | 1 (4%)                                       |
| <b>Needs improvement</b> | 2 (6%)  | 2 (7%)                                       |
| <b>Poor</b>              | 1 (3%)  | 1 (4%)                                       |

The results of using the five-level grading scale for the overall assessment of the NRLs' performance in identification of *Campylobacter* spp. are presented in Table 7. Thirty-three NRLs (27 MS-NRLs) fulfilled the criterion for excellent or good performance in identification of *Campylobacter* spp., and none scored below the acceptable limit (Table 7).

**TABLE 7.** Overall performance of 34 NRLs' sensitivity in correctly identifying *Campylobacter* spp. in proficiency test No. 40, 2025.

| <b>Grade</b>             | <b>Sensitivity</b> | <b>Combined performance in detection of <i>Campylobacter</i> spp.</b> |  |
|--------------------------|--------------------|---|--|
|                          |                    | Number (proportion) of NRLs<br>All NRLs, n=34                         | Number (proportion) of NRLs<br>MS-NRLs, n=28 |
| <b>Excellent</b>         | 95.1-100%          | 32 (94%)  | 26 (93%)                                     |
| <b>Good</b>              | 85.0-95.0%         | 1 (3%)  | 1 (4%)                                       |
| <b>Acceptable</b>        | 70.0-84.9%         | 1 (3%)  | 1 (4%)                                       |
| <b>Needs improvement</b> | 57.0-69.9%         | 0 (0%)  | 0 (0%)                                       |
| <b>Poor</b>              | < 57.0%            | 0 (0%)  | 0 (0%)                                       |

All performance parameters for detection and species identification of *Campylobacter* in chicken caecal content for all participants are presented in Table 8.

**TABLE 8.** The sensitivity (Se), specificity (Sp), and accuracy (Acc) in detecting *Campylobacter* and non-*Campylobacter* spp., the combined performance grades in detection, and the sensitivity in identification (Se id) of *Campylobacter* spp. for 34 NRLs participating in proficiency test No. 40, 2025. The performance grades in detection were based on minimum limits for sensitivity in detection of low-level samples (Se low), sensitivity in detection of high-level samples (Se high) and specificity (Sp). Green shadowed cells indicate acceptable grades: Excellent, Good and Acceptable, and red shadowed cells indicate grades below the acceptable limit: Needs improvement and Poor.

| Lab ID     | Se low | Se high | Se total | Sp   | Acc  | Performance grade in detection | Se id       |
|------------|--------|---------|----------|------|------|--------------------------------|-------------|
| <b>104</b> | 67%    | 100%    | 83%      | 100% | 89%  | <b>Acceptable</b>              | <b>100%</b> |
| <b>105</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>106</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>107</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>110</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>119</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>123</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>124</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>125</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>128</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>134</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>139</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>143</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>144</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>145</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>149</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>152</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>158</b> | 17%    | 100%    | 58%      | 100% | 72%  | <b>Poor</b>                    | <b>100%</b> |
| <b>162</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>165</b> | 83%    | 100%    | 92%      | 100% | 94%  | <b>Good</b>                    | <b>100%</b> |
| <b>167</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>169</b> | 67%    | 100%    | 83%      | 67%  | 78%  | <b>Needs improvement</b>       | <b>90%</b>  |
| <b>172</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>178</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>179</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>182</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>183</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>186</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>189</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>190</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>193</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |
| <b>195</b> | 67%    | 100%    | 83%      | 100% | 89%  | <b>Acceptable</b>              | <b>100%</b> |
| <b>197</b> | 100%   | 100%    | 100%     | 67%  | 89%  | <b>Needs improvement</b>       | <b>75%</b>  |
| <b>199</b> | 100%   | 100%    | 100%     | 100% | 100% | <b>Excellent</b>               | <b>100%</b> |

## DETECTION AND SPECIES IDENTIFICATION OF CAMPYLOBACTER IN EDUCATIONAL SAMPLES (VOLUNTARY)

The educational samples No. 29 and 30 in PT 40 were distributed to 30 NRLs and all of them reported the results of the analysis. The results of detection and species identification of *Campylobacter* in the educational samples are presented in Table 9.

Twenty-nine of the 30 NRLs detected *Campylobacter* spp. in sample No. 29. Fifteen NRLs identified one *Campylobacter* species, twelve NRLs two species, and two NRLs three *Campylobacter* species in this sample. Twenty-six NRLs identified *C. lari* and 12 *C. upsaliensis*, and 11 NRLs identified both species present in the vial. Seven NRLs identified species other than *C. lari* and/or *C. upsaliensis*: three *C. coli*, two *C. jejuni* and two *C. helveticus*.

All 30 NRLs detected *Campylobacter* spp. in sample No. 30. Eleven NRLs identified one *Campylobacter* species, whereof nine *C. jejuni*, and 19 NRLs two *Campylobacter* species, all *C. jejuni* and *C. lari*, in this sample. One NRL reported *C. coli* and one *Campylobacter* spp. which could not be identified.

TABLE 9. Results of detection and species identification of chicken caecal content samples No. 29 and 30 by 30 NRLs in proficiency test No. 40, 2025. Shadowed cells indicate correct species identification: green for correct answers regarding all species present and yellow for correct answer regarding one of the two species present in the vials.

| Sample No. | <b>Campylobacter species</b>                                  | Both <i>C. lari</i> and <i>C. upsaliensis</i> | <i>C. lari</i> (but not <i>C. upsaliensis</i> ) | <i>C. upsaliensis</i> (but not <i>C. lari</i> ) | Both <i>C. jejuni</i> and <i>C. lari</i> | <i>C. jejuni</i> (but not <i>C. lari</i> ) | <i>C. jejuni</i> (in addition) | <i>C. helveticus</i> (in addition) | <i>C. coli</i> | <i>Campylobacter</i> spp. but unable to identify species | No <i>Campylobacter</i> detected |
|------------|---|---|---|---|--|--|--------------------------------|------------------------------------|----------------|--|----------------------------------|
| <b>29</b>  | <i>Campylobacter lari</i><br><i>Campylobacter upsaliensis</i> | <b>11</b>                                     | <b>15</b>                                       | <b>1</b>  |  | 2  | 2                              | 3 <sup>a</sup>                     |                | 1  |                                  |
| <b>30</b>  | <i>Campylobacter jejuni</i><br><i>Campylobacter lari</i>      |   |   |   | <b>19</b>                                | <b>9</b>                                   |                                |                                    | 1 <sup>b</sup> | 1  |                                  |

<sup>a</sup>2 as the only reported species and 1 in addition to *C. lari* and *C. upsaliensis*.

<sup>b</sup>As the only reported species.

The overall sensitivity in detection of *Campylobacter* spp. in sample No. 29–30 was 98%. The sensitivity in detection of *C. lari* was 87% in sample No. 29 and 63% in sample No. 30. The sensitivity in detection of *C. upsaliensis* in sample No. 29 was 40%, and the sensitivity in detection of *C. jejuni* in sample No. 30 was 93%.

Eight NRLs reported both species present in the samples and no additional species not present in the vials. Since the samples were included in the PT for educational purposes, no grades were assigned for analysis of the educational samples.

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