The CDC’s National Enteric Reference and Outbreak Laboratory Quality Assurance Panel (NERO QA Panel) for the identification and subtyping of bacterial enteric pathogens

2017 Summary Report

The CDC’s National Enteric Reference and Outbreak Laboratory Quality Assurance Panel (NERO QA Panel) for the identification and subtyping of bacterial enteric pathogens is organized and conducted by the Enteric Diseases Laboratory Branch, Centers for Disease Control and Prevention (CDC). The program included four panels that assess participants’ abilities in the following areas:

*Campylobacter* (5 isolates)
  - Phenotypic identification
  - Molecular identification

*Escherichia/Shigella* (5 isolates)
  - *Escherichia*: Identification to the species level
  - For Shiga toxin producing *E. coli*:
    - O Antigen determination (top seven)
    - H Antigen determination (optional)
    - Virulence profiling
  - *Shigella*: Identification to the subspecies level
  - Serotyping (as complete as possible)
  - Virulence profiling

*Salmonella* (5 isolates)
  - Identification to the subspecies level
  - Serotyping (phenotypic and/or genetic methods)

*Yersinia* and other *Enterobacteriaceae* (2 isolates)
  - Identification to the species level

Confidential reports for each laboratory were issued via email between April and July 2017; this is a summary of the group’s performance on each of the panels. We are pleased with the participation we received in the 2017 cycle and gratefully acknowledge the support of the Public Health Agency of Canada, National Microbiology Laboratory, Winnipeg, Canada, who served as the referee laboratory.
2017 Campylobacter QA Program Summary

Fifty-four laboratories submitted results for the Campylobacter panel. The results from these laboratories are summarized below.

<table>
<thead>
<tr>
<th>Number of labs that identified the strain:</th>
<th>QA-053</th>
<th>QA-054</th>
<th>QA-055</th>
<th>QA-056</th>
<th>QA-057</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. jejuni, hippurate negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correctly the species level</td>
<td>21</td>
<td>14</td>
<td>19</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>Correctly as hippurate negative C. jejuni/coli</td>
<td>17</td>
<td>NA</td>
<td>NA</td>
<td>18</td>
<td>NA</td>
</tr>
<tr>
<td>Correctly to the genus level</td>
<td>11</td>
<td>2</td>
<td>32</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Correctly with a partial identification*</td>
<td>0</td>
<td>33</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Correctly to the genus or species level but with phenotypic, interpretation or reporting errors</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Incorrectly</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>54</td>
<td>54</td>
<td>54</td>
<td>54</td>
</tr>
</tbody>
</table>

* This includes responses such as Gram negative curved rod, Refer for identification, and reporting QA-054 as Campylobacter species or Not Campylobacter.

General Comments

- Thirty-eight labs submitted phenotypic identification results, which includes traditional phenotypic testing, MALDI-TOF testing, or a combination of traditional and MALDI-TOF testing; fourteen labs submitted a combination of phenotypic and molecular results; one laboratory submitted only molecular identification results.
- Fifty-three labs correctly reported three or more strains to either the genus or species level based on the testing performed; of those, six laboratories correctly identified all isolates to the species-level.
- The majority of the participants correctly identified or reported QA-053, QA-055, QA-056 and QA-057 to either the species or genus level. However, phenotypic testing, result interpretation and report formatting errors were seen. These included:
  - The hippurate negative strain of C. jejuni was reported as hippurate positive. The hippurate test should be developed with ninhydrin for exactly ten minutes using a timer. Longer incubation times can cause overdevelopment of the test, resulting in a false positive. The test can also be over-interpreted. A positive result should
be deep purple. A negative result can range from no color change to grey to light purple.

- Strains should be reported to the highest level of confidence of correctness and no higher. Disclaimers such as “not jejuni” or “possibly subsp. XXX” should be avoided.

- QA-054 (A. butzleri) was correctly identified to the species level by fourteen participants and to the genus level by two participants. An additional fourteen participants correctly reported this strain as “Not Campylobacter species” based on the testing performed. Some participants reported this strain as “Campylobacter species”. Although this identification is technically incorrect, it was accepted as correct for the purposes of this program because the phenotypic testing performed did not differentiate Campylobacter and Arcobacter. To differentiate between Arcobacter and Campylobacter, we recommend the following tests:
  - Aerobic growth at 36°C. Arcobacter grows aerobically at 36°C; Campylobacter does not.
  - Microaerobic growth at 25°C. As a rule, Arcobacter species grow microaerobically at 25°C. However, C. fetus and some C. hyointestinalis strains also grow under these conditions. Therefore, microaerobic growth at 25°C suggests that a strain may be Arcobacter, but it is not a definitive test.
  - MALDI-TOF based identification
  - Molecular identification methods such as 16S rRNA gene sequencing

- The public health significance of the Campylobacter species other than C. jejuni and C. coli remains unclear as we have limited species-level surveillance data for other species. Molecular tests can aid in the identification of Campylobacter species. If molecular testing is not available, ideally states should have the capacity to identify Campylobacter isolates as either C. jejuni, hippurate negative C. jejuni/ C. coli or Campylobacter species. Laboratories are always welcome to refer non-C. jejuni isolates to the CDC for full identification or confirmation.

**Phenotypic Identification**

- MALDI-TOF-based identification appears to be a useful and accurate tool for Campylobacter species. Eighteen laboratories reported performing this method and correctly identified all strains tested, except that the A. butzleri strain gave an unreliable score in one laboratory. The majority of laboratories used the Bruker MALDI-TOF instrument.

- Common phenotypic testing concerns include:
  - The use of 24 hour growth is recommended when performing phenotypic testing for Campylobacter species identification. In our experience, the use of older growth can lead to test results that are difficult to interpret or erroneous. If the growth at 24 hour is insufficient for testing, the strain should be incubated for an additional 24 hours, subcultured to fresh medium, and grown for an additional 24 hours prior to testing.
  - Selective media should not be used for routine culture of pure strains or growth for phenotypic testing because the selective agents can slow or inhibit bacterial growth of some Campylobacter which may impact the results of phenotypic tests.
- TSA II agar with sheep blood is not an optimal growth medium for *Campylobacter*. Growth is generally poor and extended incubation times may be needed to obtain sufficient growth for testing. Brain Heart Infusion (BHI) agar with 5% sheep blood is the preferred general growth medium for *Campylobacter*; it is commercially available from Fisher Scientific and Hardy Diagnostic. We are happy to provide the formulation of this medium for laboratories with in-house media preparation capacity.

- The H2S-TSI test should be performed using fresh medium prepared on the day of testing. The use of older medium can lead to unreliable results.

- The nitrate medium routinely used for testing enteric bacteria is made with a peptone basal medium which does not support the growth of all *Campylobacter* and can lead to false negative results. If using a commercially available nitrate test, ensure it is intended for use with *Campylobacter*. If preparing the medium in house, use a recipe with heart infusion basal medium as it supports the growth of all *Campylobacter* species tested to date.

- The 25°C growth test should be performed in a 25°C incubator, not at room temperature on the bench top. Room temperature can be variable and may lead to false negative results and misidentification of *C. fetus* and *Arcobacter* strains.

- Naladixic acid and cephalothin susceptibility testing should not be used for species level identification of *Campylobacter* because it no longer produces reliable results due to acquired antimicrobial resistance.
2017 *Escherichia/Shigella* QA Program Summary

Sixty-four laboratories submitted results for the *Escherichia/Shigella* panel. The results from these laboratories are summarized below.

<table>
<thead>
<tr>
<th>Number of labs correctly determining:</th>
<th>QA-058</th>
<th>QA-059</th>
<th>QA-060</th>
<th>QA-061</th>
<th>QA-062</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification of Genus and species</td>
<td>64</td>
<td>59</td>
<td>62</td>
<td>50</td>
<td>53</td>
</tr>
<tr>
<td>O antigen</td>
<td>54</td>
<td>29</td>
<td>33 (Negative for Top 7 STEC) 5 (O104)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>O:H serotype (optional)</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Stx/stx presence</td>
<td>60</td>
<td>10</td>
<td>60</td>
<td>NA</td>
<td>31</td>
</tr>
<tr>
<td>Stx/stx type (optional)</td>
<td>40</td>
<td>4</td>
<td>42</td>
<td>NA</td>
<td>21</td>
</tr>
<tr>
<td>Presence of STEC virulence gene <em>aea</em> (optional)</td>
<td>11</td>
<td>8</td>
<td>10</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Presence of STEC virulence gene <em>ehxA</em> (optional)</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Presence of invasion marker <em>ipaH</em> (optional)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>64</td>
<td>63</td>
<td>63</td>
<td>63</td>
</tr>
</tbody>
</table>

NA = not applicable

**General Comments**

- Two laboratories (3%) correctly identified and characterized all five strains with respect to genus/species, O antigen and Shiga toxin type/genotype for STEC, and genus/species (subgroup) and serotype for *Shigella*.
- Whole genome sequencing technology is advancing rapidly. Its implementation will allow public health laboratories to identify bacteria from their genome sequence and extract a variety of subtype information from these data (O:H serotype, virulence and antimicrobial resistance genes as well as a variety of sequence-based subtypes). Until this...
technology is fully implemented, we encourage laboratories to phenotypically determine the O antigens of the top seven Shiga toxin-producing *Escherichia coli* (O26, O45, O103, O111, O121, O145 and O157) and the type of Shiga toxins produced (Stx1 and Stx2) or the genes encoding these toxins (*stx*1 and *stx*2) and O antigens. We also encourage states to serotype *Shigella* isolates (determine the O antigen) because shigellosis is a nationally notifiable disease for which serotype information is collected through the Laboratory-based Enteric Disease Surveillance (LEDS) system. Serotype results are helpful in monitoring trends and detecting clusters of infection due to serotypes other than *S. sonnei*. If resources permit, please consider adding this testing to your enteric work-up.

**QA-058**
- This isolate is a typical representative of Shiga toxin-producing *E. coli* O111.

**QA-059**
- This isolate was positive for the *stx*2f gene by PCR. There is considerable nucleotide sequence variation between *stx*2f and the other *stx*2 subtypes, necessitating the detection of this gene with *stx*2f-specific PCR primers. Shiga toxin 2 was not consistently detected in this isolate using commercial toxin assays.

**QA-060**
- This isolate highlights the limitation of OK antisera and the importance of using O-specific antisera for O antigen determination. The agglutination of QA-060 observed with OK O157 antiserum, which contains antibodies against the O157 antigen and an unspecified K antigen, is likely attributable to antibodies against the unspecified K antigen because no agglutination was observed with O157-specific antiserum. Further testing with O-specific antisera revealed that QA-060 agglutinated in O104 antiserum.

**QA-061**
- QA-061: *Escherichia fergusonii* was included to assess your methods for identifying *Escherichia* species. *E. fergusonii* and *E. coli* are genetically and phenotypically similar. *E. fergusonii* can be distinguished phenotypically from *E. coli* and the other escherichiae by its ability to ferment D-adonitol, D-arabitol and cellobiose. In the near future, a newly developed genetic test, Average Nucleotide Identity (ANI), will be available through BioNumerics to facilitate the identification of *E. fergusonii* and other common enteric bacterial pathogens using whole genome sequence data.

**QA-062**
- This isolate highlights the value of using the collective phenotypic properties (biochemical and serotype results) in combination with the *ipaH* virulence gene profile to identify *Shigella*. In our hands, isolate QA-062 was unable to decarboxylate ornithine, but its other biochemical, serologic and genetic traits were consistent with *Shigella sonnei*. The ornithine decarboxylase test is an important test for differentiating *S. sonnei* from other *Shigella* species; however, serologic findings are typically prioritized over an atypical biochemical trait when assigning species in *Shigella*. While the vast majority of *S. sonnei* isolates are able to decarboxylate ornithine, occasional variants lacking this
ability do occur. In 1971, less than 1% (0.6%) of a random sampling of ~600 isolates from an unbiased collection of over 2,500 *S. sonnei* isolates received by the CDC between 1948 and 1965 were unable to decarboxylate ornithine [Ewing, WH *et al.* Biochemical Reactions of *Shigella*, DHEW Publication No. (CDC) 72-8081, 1971.]

Among isolates received more recently (2006-present), which are typically received due to atypical phenotypic traits, the percentage of *S. sonnei* isolates that were unable to decarboxylate ornithine was 6.5% (16/244). Due to the bias in CDC’s current collection, the true prevalence of *S. sonnei* isolates that are unable to decarboxylate ornithine is likely much lower than 6.5%, and there is no reason to suspect that it is not similar to that measured in 1971. Isolates like QA-062 remind us that bacteria can vary in their phenotypic properties and that one atypical phenotypic trait should not exclude a particular identification if other phenotypic and genetic characters are consistent with that identification.

**2017 Salmonella QA Program Summary**

Sixty-five laboratories submitted results for the *Salmonella* panel. Fifty-six labs performed full serotyping; those results are summarized below.

<table>
<thead>
<tr>
<th>Number of labs identifying the strain as:</th>
<th>QA-063 Baltimore</th>
<th>QA-064 Teelvekibir</th>
<th>QA-065 Senftenberg</th>
<th>QA-066 Kottbus</th>
<th>QA-067 Albany</th>
</tr>
</thead>
<tbody>
<tr>
<td>the expected serotype</td>
<td>33</td>
<td>53</td>
<td>51</td>
<td>50</td>
<td>45</td>
</tr>
<tr>
<td>a serotype other than the expected one</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>a monophasic or rough variant</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>a partial serotype</td>
<td>12</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>56</td>
<td>56</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>% expected serotype</td>
<td>59%</td>
<td>95%</td>
<td>91%</td>
<td>83%</td>
<td>80%</td>
</tr>
</tbody>
</table>

**General comments:**

- Thirty labs performed conventional serotyping and 26 performed molecular serotyping supplemented with conventional serotyping when needed.
- Overall, 25 labs (45%) correctly serotyped all five strains. Among the 280 serotype determinations in the 56 labs, 232 (83%) were the expected serotype; 26 (9%) were a serotype other than the expected one; five were monophasic variants; one was a rough variant; and, 16 strains (6%) were partially or not identified.
- Seven of the 26 strains (27%) that were not identified as the expected serotype were due
to an apparent mix-up of strains within the panel.

- Eleven labs (20%) misspelled the serotype name Senftenberg.
- Many of the partially serotyped strains were due to lack of appropriate antisera. While CDC is running out of some specificities of *Salmonella* antisera, all the reagents necessary to serotype the strains in this panel are available from CDC. Please contact us at *Salmonella@cdc.gov* for instructions on how to request *Salmonella* serotyping reagents from CDC.

**QA-063**

- Serotype Baltimore was added to the White-Kauffmann-Le Minor Scheme in the latest update. Thirty-three labs correctly identified this strain by antigenic formula, seven of which also recognized it as a newly-named serotype. The current edition (the ninth) of the scheme was published in 2007 and has been updated twice, supplement 47 in 2010 and supplement 48 in 2014. Those three documents are available electronically
  - Grimont and Weill 2007 [https://www.pasteur.fr/sites/default/files/veng_0.pdf](https://www.pasteur.fr/sites/default/files/veng_0.pdf)

  Please contact us if you need assistance in accessing any of these documents.

- Five labs identified this strain as a monophasic variant, serotype I 35:y: -. Monophasic variants can arise at any time. Based on past QA panels, it seems certain strains are more likely to be reported as a monophasic variant than other strains. This may be a characteristic of the strain or could be due to the presence of a small population of monophasic strains in the culture that was provided for the QA panel.

**QA-064**

- Routine use of genetic subtyping has reduced the importance of testing for the ancillary O antigens that vary within a serotype. Also, inconsistencies in the White-Kauffmann-Le Minor Scheme have led to the recommendation to no longer test for O6, O24, and O25. Nonetheless, some ancillary O antigens do differentiate serotypes, and we recommend continuing to test for those. We were pleased that only four labs did not test this isolate for O22 vs O23.

**QA-065**

- This isolate was included to highlight the limitations of the molecular serotyping assay. The Ht probe in the molecular assay does not react with the Ht of Senftenberg and must be confirmed phenotypically. The publication explaining this can be found here: [http://jcm.asm.org/content/49/2/565.long](http://jcm.asm.org/content/49/2/565.long)

**QA-066 and QA-067**

- O antigen 6 has been shown to be variably expressed in group O8 strains (Mikoleit 2012 [http://jcm.asm.org/content/50/12/4098.long](http://jcm.asm.org/content/50/12/4098.long)). We recommend no longer testing for O6 but including it in the antigenic formula for historic purposes and consistent nomenclature (analogous to O12). The name of the more common serotype of the O6+ and O6- serotype pair should be reported. This recommendation is discussed in a document
entitled “Revised Recommendations for the Interpretation of Salmonella Serovar”; please contact us if you would like a copy.

- About half of the labs that participated in this year’s panel tested for O6. This is somewhat higher than the proportion of labs (20 of 58 or 35%) that tested for O6 in a serogroup O8 strain in the 2016 QA Panel.

Nomenclature

- The use of non-standard antigenic formulas to represent Salmonella serotypes continues to be a problem. We understand that including all relevant antigens in the formula is not necessary for those that understand Salmonella serotyping. Given the complicated nature of Salmonella serotype designations and to try to improve understanding, we think it is important to use clear, correct, consistent nomenclature according to the White-Kaufmann-Le Minor Scheme.
  - For H antigen complexes, the primary antigen should be listed, e.g., “1,5” not “5.”
  - Antigenic factors within an antigen designation are separated by commas, e.g., g,s,t and e,n,x.
  - Flagellar antigen phases are separated by colons, e.g., I 13,23:d:e,n,z15, and not spaces, commas, or parentheses.
  - Subspecies is represented in the antigenic formula by Roman numeral, e.g., I 8,20:z4,z24:-.
  - Generic antigen formula for serotypes may contain antigens that are underlined, in square brackets ([ ]), in curly brackets ({}), or in parentheses; they denote antigenic variability within a serotype. For a strain that has been serotyped, list only the antigens that were tested for and detected in the antigenic formula. E.g., the antigenic formula for serotype Typhimurium is I 4,[5],12:i:1,2; the antigenic formula for a strain of serotype Typhimurium is I 4,5,12:i:1,2 or I 4,12:i:1,2.

Please contact us at Salmonella@cdc.gov with any questions regarding these results, to request a copy of “Revised Recommendations for the Interpretation of Salmonella Serovar”, or for information on ordering Salmonella antisera.

2017 Yersinia and other Enterobacteriaceae QA Program Summary

Sixty laboratories submitted results identifying one or both isolates for the Yersinia and other Enterobacteriaceae panel. The detailed results for the individual isolates are given below. All laboratories that submitted worksheets included phenotypic testing in their workflow, with API being the most commonly used platform. MALDI-TOF results were submitted by fourteen laboratories to supplement phenotypic testing.

QA-068

- This strain is Yersinia enterocolitica. Sixty laboratories submitted results for QA-068; all laboratories (100%) correctly identified the isolate as a Yersinia. Fifty-four laboratories (90%) correctly identified it as Yersinia enterocolitica and the remaining laboratories
identified it as “Yersinia enterocolitica/Yersinia frederksenii (1), Y. frederiksenii (1), Yersinia intermedia (1) or “Yersinia spp.” (3).

QA-069

- This strain is Cronobacter sakazakii. Fifty seven laboratories submitted results for QA-069; 34 laboratories (60%) identified the isolate as Cronobacter sakazakii. Twenty-one laboratories (37%) identified the isolate as Cronobacter spp, or Cronobacter sakazakii complex/group; two of these laboratories reported “Enterobacter sakazakii.” It is important to note that E. sakazakii equates to C. sakazakii complex, as the taxonomic revision of E. sakazakii split the former taxon into multiple species of Cronobacter. Thus, an identification of E. sakazakii with commercial panels should be reported as “Cronobacter spp.” or “Cronobacter sakazakii complex.” The C. sakazakii complex currently includes seven recognized species, of which C. sakazakii is of greatest public health concern.

Cronobacter sakazakii infections are rare but life-threatening, particularly in neonates, where it can cause potentially fatal septicemia and meningitis. Powdered infant formula has been shown to be a source of illness and outbreaks of C. sakazakii among neonates. Because of the severity of the disease and the potential for outbreaks, the CDC investigates any case of neonatal C. sakazakii. Any Cronobacter species in powdered infant formula is considered an adulterant according to the Code of Federal Regulations. The identification of any Cronobacter in a neonate should be reported to the CDC for case investigation, and the isolate forwarded for species identification and additional testing.

Identification of species within the genus Cronobacter is difficult using phenotypic testing. MALDI TOF can identify isolates to the genus level but may not distinguish among all the Cronobacter species. Differentiating C. sakazakii and its closest relative C. malonaticus is especially problematic. These two species cannot reliably be differentiated using 16S rRNA gene sequencing, but gene trees constructed from other housekeeping gene sequences clearly group Cronobacter isolates into the seven distinct species.

Yersinia, Cronobacter and other Enterobacteriaceae isolates can be sent to CDC (Unit 16) for identification as needed. The NERO has determined the performance characteristics of sequencing a segment of the rpoB gene for enteric bacterial identification. The gene segment is useful for identification of Yersinia, Cronobacter and many other Enterobacteriaceae. As the CDC and the network of public health laboratories transition to whole genome sequencing, Yersinia enterocolitica and Cronobacter sakazakii will be among the organisms for which identification and genome-based multilocus sequencing typing (MLST) will be conducted on a routine basis.