Test methodologies performed by clinical and reference laboratories for the detection of enteric bacterial pathogens such as *Salmonella* and Shiga toxin-producing *E. coli* (STEC) are rapidly changing. This document outlines recommended guidelines for enteric bacterial testing at clinical microbiology laboratories in Colorado, specifies the role of the CDPHE Laboratory Services Division in the confirmation and subtyping of suspected specimens, and provides details on what should be reported to CDPHE and which specimens should be submitted to the CDPHE laboratory.

At least 5 laboratories in Colorado have implemented one of the commercially-available multi-target enteric pathogen diagnostic assays (such as Biofire FilmArray Gastrointestinal Panel, Luminex xTAG GPP, or Prodesse ProGastro SSCS).

Current methods for outbreak detection depend on the use of isolates for subtyping (including serotyping, serogrouping, and pulsed-field gel electrophoresis [PFGE]). To maintain our very effective statewide surveillance system, it is crucial that clinical laboratories continue to report positive test findings to us and submit culture isolates or clinical material that can be used for subtyping. We strongly recommend that you continue to perform culture in order to isolate organisms from specimens that test positive using culture-independent diagnostic tests (CIDT), especially when *Salmonella*, *Shigella*, STEC, or *Vibrio* are detected. We also request that you notify us when you implement a multi-target assay or shortly before so that we can effectively support you in fulfilling your public health responsibilities.

A laboratory’s public health responsibility for enteric pathogens falls in to 2 categories: **case reporting** and **submission of clinical material** to the CDPHE laboratory.

**Reporting:**

Please continue to report all laboratory results that are positive for reportable conditions, even if no confirmatory testing is performed. Enteric bacterial pathogens that should be reported are: *Campylobacter*, *Listeria*, *Salmonella*, *Shigella*, STEC, *Vibrio* and *Yersinia*. For example, a positive Shiga toxin test, or suspected *E. coli* O157 should be reported to public health no later than 7 days after the positive test result. (The enteric parasites *Cryptosporidium*, *Cyclospora*, and *Giardia* are also reportable.)

Submission of clinical material to the CDPHE laboratory is not sufficient for case reporting because shipping could be delayed and additional data must be reported that is not included on the laboratory submission forms.

Cases can be reported in any of the following ways:

- Electronic lab reporting (for hospitals/labs currently using ELR)
• Enter the case into CEDRS (by laboratory or hospital infection control staff)
• Fax case information to the CDPHE epidemiology group at 303-782-0338. **Do not fax to the CDPHE laboratory.**
• Call the CDPHE epidemiology group at 303-692-2700.

If your lab has changed test method, remember that it is important to adjust any data exports to include positive results from the new tests. It is equally important to educate the person in charge of disease reporting at your facility so that he/she can accurately report tests and test results to CDPHE.

**Testing and submitting clinical material:**
Submission of isolates to the CDPHE laboratory facilitates timely tracking and surveillance of foodborne illness outbreaks due to bacterial pathogens. The following recommendations were developed by public health microbiologists, the Association of Public Health Laboratories, and the Centers for Disease Control and Prevention, to preserve the ability for public health to detect and contain outbreaks due to bacterial pathogens. We recommend clinical laboratories in Colorado do the following:

• Contact the CDPHE laboratory if your laboratory is considering changing methodology so that we can discuss case reporting and submission of clinical material and answer any questions you have about logistics.
• Continue to obtain and submit isolates of enteric bacterial pathogens to the CDPHE laboratory, per the CDPHE Isolate Submission Protocol, attached.
• All CIDT specimens with positive results for enteric bacterial pathogens (PCR, antigen-based, multi-analyte syndromic panels) should undergo further work-up for confirmation.
• If clinical laboratories are unable to perform reflex culture from positive CIDT specimens, the specimens should be transported in Cary-Blair transport medium or GN broth and forwarded within 24 hours to the CDPHE laboratory. If fresh stool is submitted for testing, a portion of the specimen should be inoculated into Cary-Blair transport medium and sent to the CDPHE laboratory within 24 hours. Submitting laboratories must indicate the type of testing already performed and the results of that testing on the submission form.
• Culture and isolation should be attempted for the presumptive pathogen using standard protocols for stool pathogens using a battery of selective and differential media.

Suggested plating protocol and specimen types for submission to the CDPHE laboratory:

<table>
<thead>
<tr>
<th>PCR or Antigen Positive Result</th>
<th>Recommended Media for Isolation/Inoculation (Choose one or more media type)</th>
<th>Specimen type for submission to CDPHE Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salmonella</strong></td>
<td>MacConkey&lt;br&gt;Hektoen Enteric&lt;br&gt;Xylose-Lysine-Deoxycholate</td>
<td>Isolate&lt;br&gt;Gram-Negative broth&lt;br&gt;Modified Cary-Blair transport media</td>
</tr>
<tr>
<td>Organism</td>
<td>Media/Isolate</td>
<td>Transport Media</td>
</tr>
<tr>
<td>-------------------</td>
<td>-----------------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td><em>Shigella</em> sp.</td>
<td>MacConkey Hektoen Enteric Xylose-Lysine-Deoxycholate</td>
<td>Isolate Gram-Negative broth Modified Cary-Blair transport media</td>
</tr>
<tr>
<td><em>Campylobacter</em> sp.</td>
<td>Blood Agar Campylobacter media (CAMPY-BAP, CVA, Skirrow, CSM, CCDA, or mCCDA)</td>
<td>Isolate Gram-Negative broth Modified Cary-Blair transport media</td>
</tr>
<tr>
<td>Shiga-toxin</td>
<td>Gram-Negative broth Sorbitol MacConkey</td>
<td>Isolate Gram-Negative broth Modified Cary-Blair transport media</td>
</tr>
<tr>
<td><em>Yersinia</em> sp.</td>
<td>MacConkey Cefsulodin-Irgasan-Novobiocin</td>
<td>Isolate Gram-Negative broth Modified Cary-Blair transport media</td>
</tr>
<tr>
<td><em>Vibrio</em> sp.</td>
<td>MacConkey Thiosulfate-bile salt sucrose</td>
<td>Isolate Gram-Negative broth Modified Cary-Blair transport media</td>
</tr>
</tbody>
</table>

*(Clinical Microbiology Procedures Handbook v 3)*

Because testing and subtyping of Shiga toxin-producing *E. coli* is complex, we are attaching our current guidance on handling those specimens and reporting positive results to public health. CDC has published specific guidance about testing for STEC, available at: [http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5812a1.htm](http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5812a1.htm)

We appreciate your continued efforts to maintain Colorado’s excellent surveillance system for enteric pathogens. For questions about case reporting or submission of clinical material, please contact the Communicable Disease Branch at 303-692-2700 or the Public Health Microbiology Laboratory at 303-692-3480.
STECC Workup, Reporting and Specimen Submission for Laboratories that Perform O157 Culture and Shiga toxin Testing

1. Perform Shiga toxin test and setup O157 culture.
2. If Shiga toxin test is positive:
   - Perform E. coli O157 test.
     (Can be performed at CDPHE for fee)
3. If Non-Sorbitol Fermenter (NSF) is seen on culture:
   - NSF Positive for O157?
     - Yes: Submit O157 isolate to CDPHE lab within 48 hours. Report: STEC case to state or local HD*
     - No: Stop
4. If NSF Positive for O157?
   - Yes: Submit: broth from Shigatoxin test to CDPHE lab within 48 hours. Report: STEC case to state or local HD*
     Include CDPHE “Request for Analytical Services” form with the results for tests performed at your lab written on it.
5. Stop

*Cases should be reported via the laboratory’s usual reporting mechanism (CEDRS, ELR, or fax). Call 303-692-2700 for questions about how to report a case.

March 2015

[Diagram of STEC workup, reporting, and specimen submission process for laboratories performing O157 culture only.]

*Cases should be reported via the laboratory’s usual reporting mechanism (CEDRS, ELR, or fax). Call 303-692-2700 for questions about how to report a case.
Perform Shiga toxin test.

Shiga toxin test Positive?

Yes

Submit: broth from Shiga toxin test to state lab
Report: STEC case to state or local HD*

Include CDPHE “Request for Analytical Services” form with the results for tests performed at your lab written on it.

Done

No

Stop

*Cases should be reported via the laboratory’s usual reporting mechanism (CEDRS, ELR, or fax). Call 303-692-2700 for questions about how to report a case.

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