SURVEY SUMMARY REPORT

Laboratory Methods for Identification of Non-O157 Shiga -Toxin Producing *E. coli* (STEC)

February 2008

In Collaboration with the Foodborne Diseases Active Surveillance Network (FoodNet), the principal foodborne disease component of the Centers for Disease Control and Prevention’s Emerging Infections Program
Survey Summary: “Laboratory Methods for Identification of Non-O157 Shiga-Toxin Producing E. coli (STEC)”

Thank you for participating in the June 2007 survey: “Laboratory Methods for Identification of Non-O157 Shiga-Toxin Producing E. coli (STEC).” The following document provides summary of the data collected from that survey.

Purpose of the Survey
Minnesota is one of ten states that participate in the Foodborne Diseases Active Surveillance Network (FoodNet), the principal foodborne disease component of the Centers for Disease Control and Prevention’s Emerging Infections Program www.cdc.gov/foodnet/. The success of the program is due to laboratories like yours contributing to the surveillance network. A key finding of recent surveillance has been the increase, nationally, in incidence of non-O157 Shiga-toxin producing E. coli (STEC) over the last few years. This increase is likely due to changes in testing practices across the nation.

To try to gain a better understanding of how changing laboratory practices might be affecting the increase in incidence, in June 2007 MDH, in conjunction with other FoodNet states, conducted a brief online survey regarding laboratory methods for identification of STEC. The results of the survey are found in this document.

Survey Results
A total of 138 MLS laboratories responded to the survey

PART I – Testing Methods and Future Testing Plans
The following data apply to all 138 laboratories that responded to the survey and focus on testing methods used and future plans.

E. coli O157:H7 culture practices
59/138 (43%) labs perform cultures for E. coli O157:H7
77/138 (56%) labs refer E. coli O157:H7 cultures to a reference laboratory
2/138 (1%) labs do not culture or refer out for E. coli O157:H7 testing

Among the 59 laboratories that perform cultures for E. coli O157:H7
57/59 (97%) test every stool specimen for E. coli O157:H7
1/59 (2%) culture for E. coli O157:H7 only when clinician specifically orders it
1/59 (2%) did not respond to this question

Non-culture testing methods for STEC
83/138 (60%) labs refer specimen to a reference lab for non-culture methods
9/138 (7%) labs perform non-culture methods (at the time of the survey)
45/138 (33%) do not perform or refer to a reference lab for non-culture methods
1/138 (1%) did not respond to this question

Future Plans to start using STEC non-culture testing methods
Among the 129 labs that did not use non-culture methods at the time of the survey:
88/129 (68%) do not have current plans to start using STEC non-culture methods
32/129 (25%) are currently considering starting to use STEC non-culture methods
5/129 (4%) plan to start using STEC non-culture methods in < than 6 months
1/129 (<1%) plan to start using STEC non-culture methods in > than 6 months
1/129 (<1%) did not respond to this question
2/129 (2%) answered that this question did not apply to them
PART II – Practice of the 9 Labs that use STEC non-culture methods
The following answers focus on the practices of the 9 laboratories that reported using non-culture methods at the time of the survey.

Use of STEC non-culture methods alone or in combination with culture for *E. coli* O157:H7

7/9 (78%) labs use both STEC non-culture method and culture for *E. coli* O157:H7
4/7 labs performed both on all stool specimens
2/7 labs use culture for *E. coli* O157:H7 on all stools, and STEC non-culture method for STEC on some stool specimens
1/7 labs use STEC non-culture method, but also refer out for *E. coli* O157:H7 culture when specifically requested
2/9 (22%) labs use STEC non-culture method and do not culture for *E. coli* O157:H7

After the survey was completed, MDH received notification that two additional laboratories are now using non-culture methods. Both plan to continue culturing for *E. coli* O157:H7.

STEC Non-culture Test Kit Used

3/9 (33%) labs use Immunocard STAT! EHEC (Meridian Biosciences)
3/9 (33%) labs use Premier EHEC (Meridian Biosciences)*
1/9 (11%) lab uses Immunocard STAT! *E. coli* O157 Plus (Meridian Biosciences)
1/9 (11%) lab uses Biostar OIA Shigatox
1/9 (11%) lab uses ProSpecT Shiga toxin microplate assay (Remel)

* will be changing to Immunocard STAT! EHEC (Meridian Biosciences) soon

When did the laboratories start using a non-culture method for STEC testing?

<table>
<thead>
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<th>Year started using non-culture</th>
<th>Number of laboratories</th>
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Additional Information on *E. coli* O157 and non-O157 STEC

Preliminary 2007 STEC surveillance data for Minnesota
In 2007 MDH recorded 229 STEC cases in MN
165/229 (72%) serotyped as *E. coli* O157
43/229 (19%) serotyped as *E. coli* non-O157*
21/229 (9%) no isolate available for serotyping

* of the 43 *E. coli* non-O157, the 3 most common serogroups were
O111 (10 cases)
O26 (10 cases)
O103 (9 cases)
32 of the 229 STEC cases were identified using non-culture methods
10/32 (31%) serotyped as *E. coli* O157
18/32 (56%) serotyped as *E. coli* non-O157
4/32 (13%) no isolate received therefore not able to confirm or serotype

*2 broths (not included in the totals) were positive for STEC at referring laboratory, but upon receipt at MDH-PHL could not be confirmed by PCR or culture.*

**Clinical Significance of O157 and non-O157 STEC**

In general, illnesses caused by STEC range from mild non-bloody diarrhea to severe bloody diarrhea and HUS; in addition, abdominal cramps and the lack of a high fever are common clinical features. HUS is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure.

While O157 and non-O157 STEC are isolated from diarrhetic stools in approximately equal numbers, serotype O157 is thought to be significantly more important from both medical and public health standpoints, although there is a paucity of hard data on this subject. Most cases of HUS, and most outbreaks caused by STEC appear to be associated with serotype O157. Research is ongoing to determine better markers for serious Shiga toxin-related disease. A good summary can be found in the following recent presentation by Dr. Patricia M. Griffin, M.D., Chief of CDC Enteric Diseases Epidemiology Branch, “CDC Perspective on non-O157 Shiga toxin-producing *E. coli* (STEC) in the United States” [http://www.fsis.usda.gov/PPT/Non-0157_STEC_Griffin.ppt](http://www.fsis.usda.gov/PPT/Non-0157_STEC_Griffin.ppt) (1,2)

**Recommendations for Laboratory Testing**

There has been considerable confusion in our community about STEC testing methods, which has been compounded by aggressive marketing of Shiga toxin tests. Until tests are available which better predict STEC virulence, O157 culture should remain the core STEC testing strategy. O157 culture is relatively simple, especially when CT-SMAC or other selective media designed for O157 are used, and fits well into a standard *Salmonella/Shigella/Campylobacter* protocol. Optimally, a Shiga toxin-specific test should be run in conjunction with O157 culture, as non-O157 STEC, while less significant than O157, are nevertheless recognized as pathogens and a rare cause of HUS. Alternately, O157 culture negative specimens from cases where STEC is strongly suspected may be sent to MDH-PHL for Shiga toxin testing. Finally, for facilities that are using Shiga toxin tests as a primary screening tool, the clinical laboratory protocol should include reflex to O157 culture upon positive STEC findings and at a minimum, referral of clinical materials to MDH-PHL for further characterization.

**Primary plating of specimen:** The specimen should be plated onto MacConkey Sorbitol (SMAC) agar in order to isolate and differentiate the *E. coli* O157:H7. Another useful medium is MacConkey Sorbitol agar supplemented with cefixime and tellurite (CT-SMAC), which inhibits non-O157 *E. coli*, greatly reducing labor, and increasing test sensitivity.

**Serotyping of isolates:** Sorbitol negative isolates should be identified biochemically as *E. coli*, and then serologically tested for O157 and H7 antigens. It is important to note that other species may cross-react with O157 antiserum or latex reagents, so accurate identification of *E. coli* is important. If your lab does not perform serotyping, the isolate should be sent to the MDH-PHL or another reference lab for testing.

**Antimicrobial Susceptibility:** Retrospective studies suggest that patients who received antibiotics may be at greater risk of developing HUS. As such, antibiotic testing is contraindicated for patient management of gastro-intestinal *E. coli* O157:H7.
Reportable Disease Rule
If hemolytic uremic syndrome (HUS) is suspected, it must be reported ‘immediately by phone’ to MDH – Acute Disease Investigation and Control Section. In addition, submission of a specimen or clinical materials* to MDH-PHL is required on all patients suspected or diagnosed with HUS. This poses a challenge to many laboratories that are not provided diagnosis information however it is extremely important in HUS cases. The Communicable Disease Reporting MN Rule, 4605.7040, can be found at: http://www.health.state.mn.us/divs/idepc/dtopics/reportable/rule/rule.html

Submit specimens as isolates in pure culture, broth of specimen, or properly preserved original stool specimen. Grow cultures for 18-24 hours and submit as soon as possible. Plates or slants are both acceptable. Call the MDH Enterics Laboratory (651-201-5048) with any questions.

*Clinical materials: Submit isolate or, if an isolate is not available, submit material containing the infectious agent in the following order of preference: a patient specimen; nucleic acid; or other laboratory material.

REFERENCES

Thank You
MDH sincerely appreciates all 138 laboratories that provided data for this survey. Your input is important for the understanding of STEC surveillance data. If you have any questions or comments regarding this survey summary or STEC testing, please do not hesitate to contact us.

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