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Identification of *Escherichia coli* O157:H7 in a Proficiency Testing Program

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- This report presents the results of a study to assess whether a nationwide proficiency testing program can evaluate laboratories' ability to detect and identify *Escherichia coli* O157:H7.
- The results suggest that infection with *E. coli* O157:H7 and other Shiga toxin-producing *E. coli* (STEC) strains is often undiagnosed because many laboratories lack procedures and protocols to reliably detect these organisms.
- To correct this problem, laboratories should review and update their practices in 3 areas: policies regarding which stool specimens to screen for *E. coli* O157:H7 and other STEC strains, procedures for isolating and identifying these organisms, and mechanisms for informing physicians about stool culture practices.

First described in 1982, *Escherichia coli* O157:H7 is now recognized as a significant cause of food borne and waterborne illness in the industrialized world. Each year, *E. coli* O157:H7 and other Shiga toxin-producing *E. coli* strains (STEC) cause an estimated 73,000 cases of hemorrhagic colitis and 60 deaths in the United States.[1,2] As many as 8% to 18% of victims go on to develop hemolytic uremic syndrome (HUS).[1-3] These patients may require kidney dialysis and transfusions, and some are left with chronic renal failure and neurological damage; 3% to 5% of patients with HUS die.[2,4]

The greatest threat to public health from *E. coli* O157:H7 is from unintentional contamination of food or water, but contamination could also be deliberate. This happened in 1984, when members of a religious sect sprayed salad bars at 2 restaurants in Oregon with *Salmonella enterica* serovar Typhimurium, sickening 750 people.[5] The terrorist attacks on the World Trade Center on September 11, 2001 and the anthrax attacks in October 2001 heightened concern that the food or water supply might be deliberately tainted, prompting the Centers for Disease Control and Prevention (CDC) and its partners to develop a list of biologic agents that could be used in an attack. *E. coli* O157:H7 appears on this list as a biothreat level B agent (which means it has a moderate ease of transmission, moderate morbidity, and low mortality).[5]

Whether contamination of the food or water supply occurs accidentally or deliberately, clinical laboratories play a key role in the detection and surveillance of outbreaks.[5] To protect the public health, it is critical that they are able to identify or rule out pathogens such as *E. coli* O157:H7. However, surveys have shown that laboratories vary widely in their stool culture protocols and their ability to reliably isolate and correctly identify this organism.[6,7]

In 2003, American Proficiency Institute (API) and the Michigan Department of Community Health, with a grant from the CDC, conducted a study to evaluate whether a nationwide proficiency testing program could assess laboratories' ability to detect *E. coli* O157:H7. This report presents the results of that study.

Materials and Methods

To prepare laboratories for the proficiency testing event that would provide data for this study, API included an educational commentary on *E. coli* O157:H7 in its 2002 2nd Test Event Participant Data Summary. This commentary explained how to isolate and identify *E. coli* O157:H7 and stressed the importance of developing screening protocols that meet public health, patient care, and economic needs.

Data was acquired from API's 2003 1st Test Event (March 2003). A KWIK-STIK™ sample (Sample SC-01, prepared by MicroBioLogics, St. Cloud, MN) containing *E. coli* O157:H7 was distributed to 420 laboratories enrolled in the API Comprehensive Bacteriology Program. These laboratories represent clinics, private laboratories, public health laboratories, and hospitals with bed sizes of 25 to 300. Participants were told that the sample was a stool culture specimen with a physician request to screen for *E. coli* O157:H7. A questionnaire about stool culture practices was included as a separate page not tied to performance evaluation.

Results were processed by API and assigned a performance grade based on criteria developed by the Centers for Medicaid Studies and published in the *Federal Register* on February 28,1992. Statistical analyses were done with proprietary software developed at API.

Results

Of the 420 laboratories enrolled in the Comprehensive Bacteriology Program, 243 provided a response to Sample

SC-01 (**Table 1**). Of these, 128 (53%) correctly identified the organism as *E. coli* O157:H7 (4%), *E. coli* O157 (12%), presumptive *E. coli* O157 (35%), or *E. coli*, non sorbitol-fermenting (2%). However, 66 laboratories (27%) incorrectly reported "no stool pathogens isolated" even though the testing instructions indicated the physician's request to screen for *E. coli* O157:H7; and 8 (3%) erroneously identified the organism as "*E. coli*, not O157:H7."

Of the 243 laboratories that reported a result for Sample SC-01, 215 returned the "Stool Culture Practices Survey." The first question, "Does your laboratory include screening for *E. coli* O157:H7 in stool cultures?" was answered "Yes" by 122 (57%) of the respondents and "No" by 93 (43%) of the respondents. Those who indicated they screen for *E. coli* O157:H7 answered the remaining questions about their laboratory's stool screening protocols and procedures (**Table 2**). Of 215 respondents, 106 (49%) indicated they screen at least all bloody stool specimens for *E. coli* O157:H7 (20 laboratories screen only bloody stool specimens, and 86 laboratories routinely screen all stool specimens).

Discussion

In this study, both the percentage of laboratories screening for *E. coli* O157:H7 (57%) and the percentage of laboratories screening at least all bloody specimens (49%) are significantly lower than the results obtained in a similar survey of laboratories in FoodNet sites from 1995-2000 (95% and 84%, respectively).[7] Instead, the data correlate more closely with a 1994 nationwide random survey of microbiology laboratories throughout the United States which showed 54% screened at least all bloody stools.[6] These differences may reflect a higher awareness of current recommendations regarding screening protocols in laboratories associated with FoodNet surveillance. The results of our study suggest that12 years after a recommendation by the Association of State and Territorial Public Health Laboratory Directors to screen at least all bloody stools for *E. coli* O157:H7,[6] many laboratories still fail to do so. This, along with the fact that 30% of respondents failed to correctly identify *E. coli* O157:H7 in the survey sample, implies that many laboratories lack protocols to ensure reliable detection of this organism. To correct this problem, laboratories should examine and update their practices in 3 areas: policies regarding which stool specimens to screen for *E. coli* O157:H7 and other O157 strains, procedures for isolating and identifying these organisms, and mechanisms for informing physicians about stool culture practices.

The issue of which stool specimens to screen for *E. coli* O157:H7 and other O157 strains has been controversial, but the current recommendation is to screen all stool specimens submitted for culture of bacterial enteric pathogens.[1,7] The practice of screening only bloody stools is problematic because the determination of whether diarrhea is bloody cannot always be made by examining the specimen.[7] The practice of screening only upon physician request is also insufficient because many physicians erroneously believe the laboratory routinely screens for *E. coli* O157 and therefore often do not specifically request screening.[8]

The 2 most common reasons given for not routinely screening specimens for *E. coli* O157 are that the local incidence is too low or that the cost of screening is too high.[6] The perception that the local incidence of *E. coli* O157:H7 is low may well be false because surveys have consistently shown a greater incidence of *E. coli* O157:H7 in areas of the country with higher screening rates.[6,7] Although the cost of screening does add to the cost of performing a stool culture, this expense must be weighed against the expense of failing to correctly diagnose this infection. Patients infected with *E. coli* O157:H7 have undergone unnecessary exploratory surgeries, colonoscopies, barium enemas, and appendectomies.[6] Also, failure to quickly diagnose this infection could make it more difficult and costly to manage an outbreak associated with contaminated food or water.

To screen for *E. coli* O157:H7 and other O157 strains, laboratories should at least plate stool specimens on Sorbitol-

Table 1_Participant Responses for API Sample SC-01, 2003 1st Test Event

Participant Response	Number	(%) of Laboratories
E. coli O157:H7	9	(4)
E. coli O157	29	(12)
Presumptive E. coli O157	85	(35)
E. coli, not O157	8	(3)
E. coli, non sorbitol-fermenting	5	(2)
Growth, referred for ID	38	(16)
No stool pathogens isolated	66	(27)
Other response, acceptable	3	(1)
Total	243	(100)

Table 2_Responses to Stool Culture Practices Survey

Survey Question	Yes	No	Blank
Does your laboratory include screening for *E. coli* O157:H7 in stool cultures?*	122	93	0
• All stool specimens?	86†	28	8
• Only upon physician request?	19†	63	40
• Only bloody specimens?	20†	63	39
Does your *E. coli* O157:H7 procedure include using sorbitol MacConkey agar?	112	8	2
Does your lab perform serotyping for *E. coli* O157 on site?	36	84	2
Does your lab test for the production of Shiga-like toxins if your isolate is nonmotile or tests negative for the H7 antigen?	5	113	4
Does your lab report all positives to your state lab?	110	5	7
Does your lab send the organism to your state lab for epidemiology?	91	22	9

**Only laboratories that screen for* E.coli *O157 answered the remaining questions.*
†Three laboratories answered "yes" to more than 1 question.

MacConkey agar (SMAC) and examine for growth of non sorbitol-fermenting colonies. Non sorbitol-fermenting colonies should then be further tested, either on site or at a state or reference laboratory. Confirmation that a non-sorbitol-fermenting organism is a strain of *E. coli* O157 requires 2 steps: detection of the O157 antigen with O157 antiserum or latex reagent and biochemical confirmation that the organism is *E. coli*.[1,6] Definitive identification as *E. coli* O157:H7 requires further testing for the H7 antigen; most laboratories use a reference laboratory for this step.

Although the focus of this study has been laboratories' ability to detect and identify *E. coli* O157:H7, the emerging opinion is that laboratories should develop criteria to screen for other STEC serogroups as well.[1,3] This is because, although *E. coli* O157:H7 and other O157 strains cause most cases of hemorrhagic colitis, other STEC serogroups are increasingly implicated.[1] Until recently, it was not practical to screen for non-O157 serogroups because plating specimens on SMAC will not detect these organisms. However, several methods (including latex agglutination and enzyme immunoassay) are now available to directly detect Shiga toxins in stool specimens.[1,3] Consequently, testing stool specimens for the presence of Shiga toxins is now recommended as a method to screen for all STEC strains.[1,3]

Finally, informing physicians about stool culture practices is crucial to ensure detection of *E. coli* O157. A survey comparing physicians' beliefs about laboratory stool culture practices to actual practices reported by the laboratories showed that most physicians either did not know their laboratory's stool culture protocol or mistakenly assumed the laboratory routinely screened all specimens for *E. coli* O157 strains.[8] As a direct result of this misunderstanding, many specimens from patients with bloody diarrhea were not screened for *E. coli* O157.[8] To avoid confusion, the laboratory report should explicitly state the organisms for which the stool was examined.[8-10]

Conclusion

The results of this study support the contention of many public health officials that infection with *E. coli* O157:H7 and other STEC strains continues to be underreported and misdiagnosed. Solving the problem will require the coordinated efforts of clinical laboratories, physicians, and public health officials. Education and surveillance through laboratory proficiency testing programs can contribute to this effort by raising awareness of critical issues in protecting the public health. LM

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