

Identification of *Escherichia coli* O157:H7 in a Proficiency Testing Program: An Update of Laboratory Performance

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Abstract

Objective: By analyzing and comparing results from a 2008 proficiency test (PT) event to results from a 2003 PT event, we assessed whether laboratories' abilities to detect *Escherichia coli* (*E. coli*) O157:H7 had improved in the intervening 5 years.

Methods: A proficiency test sample containing *E. coli* O157:H7 was mailed to participants

enrolled in a PT program, and a survey about stool culture screening practices was distributed to laboratories that had submitted a result for the proficiency test sample.

Results: In 2008, 85.3% of laboratories detected *E. coli* O157:H7, compared to 70% of laboratories in 2003. Also, 72.3% of laboratories now screen at least all bloody stools, compared to 49% of laboratories in 2003.

Conclusions: Laboratories' abilities to detect *E. coli* O157:H7 has improved substantially since 2003. However, many laboratories still fail to follow screening recommendations for Shiga toxin-producing *E. coli*.

Clinical laboratories play a key role in the identification and surveillance of pathogens implicated in food borne illnesses. To protect the public health, they must be able to quickly identify or rule out pathogens which may be the cause of an outbreak. One such pathogen is *Escherichia coli* (*E. coli*) O157:H7, a strain of Shiga toxin-producing *E. coli* (STEC) which was first recognized in 1982. However, surveys have shown that laboratories vary widely in their stool culture protocols and in their abilities to detect this organism.^{1,2}

In 2003, the American Proficiency Institute (API) and the Michigan Department of Community Health, with a grant from the Centers for Disease Control and Prevention (CDC), conducted a study to assess laboratories' abilities to detect *E. coli* O157:H7 in a proficiency test (PT) sample.³ This study yielded 2 disturbing results. First, 30% of the participants failed to detect *E. coli* O157:H7, even though they were explicitly instructed to test for this organism. Second, only 49% of respondents followed a recommendation by the Association of State and Territorial Public Health Laboratory Directors to screen at least all bloody stools for *E. coli* O157:H7.³

Because the ability to detect *E. coli* O157:H7 is vital to protecting public health, it is important to know whether laboratories have updated their protocols and improved their practices. To this end, in 2008 we re-examined laboratory performance in detecting *E. coli* O157:H7 in a PT sample. The following is a report of our findings.

Materials and Methods

Data for this study were acquired from API's 2008 Third Test Event. A PT sample (Sample SC-02, prepared by Gibson Laboratories, Lexington, KY) containing *E. coli* O157:H7,

Citrobacter freundii, and *Enterococcus faecalis* was distributed to 746 laboratories enrolled in the API Comprehensive Bacteriology Program. The survey population consisted mostly of laboratories in hospitals with fewer than 400 beds. Other participants included independent laboratories, clinic laboratories, physician office laboratories, ambulatory surgical centers, and point-of-care testing laboratories. Of the 746 laboratories enrolled in the Comprehensive Bacteriology Program, 467 returned a result for Sample SC-02.

Participants were instructed to reconstitute Sample SC-02 in the rehydration fluid provided and then test it for *E. coli* O157:H7, *Salmonella*, *Shigella*, and *Campylobacter* according to their laboratories' stool culture protocol. Participants that did not routinely test for a particular pathogen were asked to note this on the result form. Results were processed by API and assigned a performance grade based on criteria developed by the Centers for Medicare and Medicaid Services.

After the results were tabulated, a survey about stool culture practices was faxed to 460 laboratories that had tested sample SC-02. (The remaining 7 laboratories had not provided API with fax numbers.) The survey questions were designed to elicit information about whether laboratories followed current recommendations for screening, testing, and reporting results for *E. coli* O157:H7 and other STEC. A total of 292 surveys were returned.

Results

Of the 467 laboratories that tested Sample SC-02, 387 (82.9%) reported a result for *E. coli* O157:H7 (Table 1). Of these, 330 (85.3%) correctly detected this pathogen and 57 (14.7%) failed to detect it.

Table 1 Participant Responses to Proficiency Test Sample SC-02

Response	No. (%) of Participants
<i>Acceptable</i>	
<i>E. coli</i> O157	82 (21.2%)
<i>E. coli</i> O157:H7	30 (7.8%)
Non-sorbitol fermenting <i>E. coli</i>	46 (11.9%)
Presumptive <i>E. coli</i> O157	150 (38.8%)
Growth-referred for ID	22 (5.7%)
<i>Unacceptable</i>	
No <i>E. coli</i> O157 isolated	19 (4.9%)
No stool pathogens isolated	38 (9.8%)
Total	387 (100.0%)

Of the 80 (17.1%) participants that did not report a result for *E. coli* O157:H7, 69 indicated they did not test for this organism. The remaining 11 participants failed to indicate they did not test for *E. coli* O157:H7. However, because they did not report a result for *E. coli* O157:H7, we assumed they did not routinely test for this organism.

On the stool culture practices survey, 244 (83.6%) of 292 respondents indicated they screen stool specimens for *E. coli* O157:H7 or other STEC. These respondents answered the remaining questions about screening protocols, testing procedures, and reporting practices. **Table 2** summarizes the results of this survey.

Table 2 Responses to Stool Culture Practices Survey*

Survey Question	No. Responding "Yes"
Do you screen for <i>E. coli</i> O157:H7 or other STEC?	244
Do you screen ... †	
all stool specimens?	202
at least all bloody specimens?	100
only upon physician request?	30
only bloody specimens?	9
only pediatric specimens?	4
Do you use sorbitol-MacConkey agar?	201
Do you confirm a non-sorbitol fermenter is <i>E. coli</i> ?	175
Do you test suspicious colonies for ...	
O157 antigen?	64
H7 antigen?	39
Do you test for Shiga toxin ...	
from colonies (sweeps or isolated)?	33
in feces or enrichment broth?	67
Do you test for O157 antigen ... ‡	
from colonies (sweeps or isolated)?	38
directly from feces?	12
from feces in enrichment broth?	16
Does your laboratory report specify the pathogens for which you screen?	196

*292 laboratories returned the Stool Culture Practices Survey.

†Some respondents provided more than 1 answer to this question.

‡This question specified immunoassay methods.

Discussion

The results of the 2008 PT event and the responses to the 2008 survey questions suggest that, since 2003, substantially more laboratories now routinely screen for *E. coli* O157:H7 and substantially more laboratories can detect it. In 2003, only 49% of the laboratories surveyed indicated they routinely screened at least all bloody stool specimens for *E. coli* O157:H7.³ In contrast, responses to the 2008 Stool Culture Practices Survey indicate at least 72.3% of laboratories performing stool cultures now screen at least all bloody specimens. This includes 202 (69.2%) that screen all stool specimens and 9 (3.1%) that screen only bloody specimens. Similarly, in 2003, 30% of laboratories that tested the PT sample failed to detect *E. coli* O157:H7.³ In contrast, only 14.7% of laboratories that tested the PT sample in 2008 failed to detect this organism. Despite these improvements however, 5 significant concerns remain.

First, although the percentage of laboratories screening for *E. coli* O157:H7 has increased and PT performance has improved, a substantial number of laboratories performing stool cultures still either cannot reliably detect this organism or do not look for it. In the 2008 PT event, only 330 (70.7%) of 467 laboratories provided an acceptable result for sample SC-02. The remaining 137 (29.3%) laboratories either reported an unacceptable result or failed to screen for *E. coli* O157:H7.

Second, many laboratories that do test for *E. coli* O157:H7 lack screening policies to optimize detection of this pathogen. For example, on the 2008 Stool Culture Practices Survey, 4 (1.6%) respondents indicated that they screen only pediatric specimens, and 30 (12.3%) indicated that they screen only upon physician request. The practice of screening only upon physician request is problematic because physicians may erroneously believe the laboratory routinely screens for *E. coli* O157:H7 and a specific request is not needed.⁴ Moreover, there is consensus that laboratories should screen all stools, as evidenced by the CDC's recommendation in 2009 that laboratories include STEC in their routine enteric panel (along with *Salmonella*, *Shigella*, and *Campylobacter*).⁵

Third, many laboratories using a sorbitol-MacConkey (SMAC) plate to screen for *E. coli* O157:H7 apparently fail to confirm a non-sorbitol fermenting isolate is *E. coli*. On the 2008 Stool Culture Practices Survey, only 175 (71.7%) of the respondents screening for *E. coli* O157:H7 indicated they confirm that non-sorbitol fermenters are *E. coli*. Failure to confirm a non-sorbitol fermenting isolate is *E. coli* could lead to a false positive report of *E. coli* O157, because strains of several bacterial species can cross react with O157 antiserum.^{6,7}

Fourth, many laboratories apparently fail to explicitly report the pathogens for which they screen. On the 2008 Stool Culture Practices Survey, only 196 (80.3%) respondents indicated their laboratories' reports specified the pathogens for which they screen. The true percentage of laboratories reporting pathogens for which they screen may well be lower, because only laboratories that routinely screen for *E. coli* O157:H7 answered this question on our survey. Failure to name the pathogens could leave clinicians with the false impression that the laboratory tested for *E. coli* O157:H7 and the result was negative. To remedy this, the College of American Pathologists (CAP) now requires that reports from laboratories it inspects name all pathogens included in screening.⁸ However, most laboratories in our study are inspected by

agencies other than the CAP, and these agencies may not require reports to name pathogens for which laboratories screen.

Finally, many laboratories may not follow CDC recommendations to optimize detection of both *E. coli* O157:H7 and other STEC, a concern that has been noted by other researchers.⁹ Shiga toxin-producing *E. coli* strains other than *E. coli* O157:H7 are increasingly being implicated in disease outbreaks. These non-O157 STEC strains cannot be detected by culture with SMAC agar. For this reason, the CDC now recommends that, in addition to culture with SMAC, laboratories screen all stool samples for Shiga toxins or the genes encoding them with non-culture methods such as enzyme immunoassay (EIA) or polymerase chain reaction (PCR).⁵ However, several respondents to our Stool Culture Practices Survey commented they had discontinued or were considering discontinuing culturing with SMAC to screen exclusively for Shiga toxin. This is controversial for 4 reasons. First, EIA is less sensitive than SMAC screening for *E. coli* O157:H7, the STEC strain most often encountered.¹⁰ Second, screening by EIA alone can occasionally yield false-positive results.⁵ Third, failure to culture or a delay in culturing could adversely impact patient care.¹⁰ Finally, delay in culturing could impede recognition of an outbreak and hinder efforts to identify the source.^{5,10}

Conclusion

The results of this study are encouraging because they imply, since 2003, the number of laboratories screening for *E. coli* O157:H7 and other STEC has increased substantially. The responses to the 2008 PT sample also indicate that more laboratories can detect *E. coli* O157:H7 when it is present; however, there is still much room for improvement. In particular, microbiology supervisors should ensure their practices reflect current CDC screening recommendations and laboratory reports specify pathogens for which the laboratory

screens. Also, to ensure that laboratories comply, accrediting agencies should include recommended screening and reporting practices in inspection checklists. LM

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