Diagnosis of Shiga Toxin-Producing *E. coli* (STEC) Infections: A Multi-Faceted Approach

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Background

- *E. coli* that produce Shiga toxin (Stx) = STEC
  - 150 different serotypes associated with illness
    - O antigen = lipopolysaccharide
    - H antigen = flagellar
    - *E. coli* O157:H7 most commonly reported
- Toxins similar structure and function to toxins produced by *Shigella dysenteriae* type 1
  - Stx1 and Stx2
  - 95% of O157 strains produce Stx2
  - 40% of non-O157 STEC produce Stx2
Epidemiology

- STEC causes ~100,000 illnesses/yr, 3200 hospitalizations, 91 deaths

- Approximately 8% of people with O157 STEC develop hemolytic uremic syndrome (HUS)
  - Thrombocytopenia
  - Hemolytic anemia
  - Renal failure
Transmission

- Occurs year round but especially summer
- Occurs in persons of all ages, but most common in children <5 yr and risk for HUS highest in this age group
- Occurs by consumption of undercooked ground beef, unpasteurized juice, raw milk and produce (spinach), contaminated water
- Low infectious dose (<100 O157 organisms)
Approach to STEC Testing Varies

1. Test all stools submitted for bacterial diarrhea
2. Test bloody stools only
3. Test only stools from children
4. Test stools only in warmer months
5. Test only with a specific physician’s order
6. Test if an outbreak has been reported
7. Combination of criteria 2, 3, 4, 5
STEC: The Iceberg Effect

Detected

Infected
If You Look, You Will Find
STEC Infections Are Not Rare Events

Incidence of Laboratory-Confirmed Infections at Selected FoodNet Sites, 2007
What the Physician Needs to Know

- Not just seasonal - occurs all year round
- Not just bloody diarrhea – miss significant # if rely on clinical lab observing blood
- Not just children – lower incidence in >65 y.o. (9% vs 27% in <10 yr) but more hospitalized
- Not just O157 - half of the cases are non-O157
- Must act quickly - only 3 - 4 days to prevent HUS
Laboratory Testing Strategies for STEC

1. Culture only
2. Non-culture toxin assay, followed by culture on toxin positives
3. Non-culture toxin assay, send toxin positive broths to Public Health Lab
4. Simultaneous culture and non-culture toxin assay
5. PCR test (not current practice)
Isolation Media to Recover *E. coli* O157

- SMAC
- CT-SMAC
- CHROMagar O157
- Rainbow Agar O157
Culture and Identification of *E. coli* O157:H7 in Clinical Lab

1. **Fecal Specimen**
2. **Select colony**
3. **Agglutination in O157 antiserum**
4. **Biochemically identify as *E. coli***
5. **Isolate to public health lab**

Confirm O157 within 24 hr to direct patient care
Isolate available for typing by PHL
Misses sorbitol-fermenting O157
Misses non-O157 STEC
Importance of Detecting Non-O157 STEC Infections

• In non-outbreaks studies of STEC cases
  – 46% non-O157 STEC
  – 54% O157 STEC
• Global hot spots where non-O157 more important
  – Germany, Australia, Argentina
  – 36% of patients with HUS lacked blood in stool

Johnson et al. CID 43: 1587, 2006
1,945 Isolates of Non-O157 STEC Serogrouped by CDC, 1983-2005

510K-approval of commercial Shiga toxin EIA

**CDC, unpublished data**
Classic: Cell Cytotoxicity Assay

- Vero (African green monkey kidney) and HeLa cells are sensitive to Stx
- Use sterile filtrate from fresh or Cary-Blair preserved stools inoculated onto cells and observed for CPE
  - Neutralize CPE with specific antibody to Stx
- TAT 48-72 hr
- Research tool
Rapid Tests to Detect Stx1 and Stx2

- Premier EHEC
  - stool, broth, isolate
- ProSpecT Shiga Toxin
  - stool, broth
- Duopath Verotoxin GLISA
  - colony sweeps
- ImmunoCard STAT! EHEC
  - broth
<table>
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<th>Sample</th>
<th>Premier EHEC (cytotoxin assay standard)</th>
<th>Prospect STEC (cytotoxin assay standard)</th>
<th>Immunocard STAT! EHEC (Premier EHEC assay standard)</th>
<th>Duopath Verotoxin (Premier EHEC assay standard)</th>
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<td>92%</td>
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<td>Cary Blair</td>
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<td>Not stated</td>
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<td>99%</td>
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<td>Sweeps of Agar Growth</td>
<td>93%</td>
<td>NA</td>
<td>100%</td>
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</table>
Outbreak of STEC serotype 045 in state correctional facility that would not have been found without EIA; confirmation by PCR for stx1

Pseudo-outbreak in day care center based on positive EIA resulted in inappropriate and unnecessary public health action; actual cause norovirus
Non-O157 STEC Diagnosis in the Clinical Lab

Stool Specimen → Enrichment broth → STEC Diagnostic Assay → Public Health Lab

Culture for O157, send colony or Shiga toxin-positive broth to public health lab

All serotypes of STEC detected with 24 hr
Delays detection and isolation of O157
Misses O157 not actively expressing toxin
Recommendations for Diagnosis of Shiga Toxin–Producing Escherichia coli Infections by Clinical Laboratories

- Authored by representatives from CDC, APHL, ASM, public health labs, commercial reference labs, academic medical centers
- Aimed at clinical and public health labs and the clinicians
- 97 references to support recommendations
Best Practice Recommendation

- All stools submitted for diagnosis of community-acquired diarrhea (Salmonella, Shigella, Campylobacter) should also include culture for E. coli O157:H7 and toxin assay for non-O157 STEC
- Simultaneous culture for O157 STEC and toxin assay for STEC
- Prompt reporting of STEC to physician
- Forward all positives to public health lab ASAP
Algorithm for STEC Detection: Clinical Labs

Stool Specimen

- **Broth**
  - 18-24 hrs
  - Shiga toxin immunoassay
  - If no O157 found in broth:
    - Stx⁺ broth to PHL

- **O157 culture (CT-SMAC)**
  - 18-24 hrs
  - Sorbitol non-fermenting colonies
    - ID as *E. coli* or test for Stx
  - O157 latex reagent
  - O157 isolate to PHL
The Role of Public Health Labs in the Diagnosis of STEC

- Timely confirmation
- Timely entry into PulseNet
- Feedback to the submitting lab
- Treat toxin + like culture + STEC
- Confirm non-O157 with antisera
- Prompt diagnosis and submission to PHL allows for timely public health action
Rapid Reporting to PHL and *E. coli* O157 Outbreak in Contaminated Spinach

- 9/08/06 – WI PHL reported cluster of *E. coli* O157
- 9/12/06 – CDC PulseNet confirmed PFGE patterns match patient isolates from other states
- 9/13/06 – OR and WI alerted CDC spinach as the suspected source
- 9/14/06 – 8 states reported 50 cases
- 9/14/06 PM – FDA banned bagged spinach
- 206 cases from 26 states
  - 70% female
  - 51% hospitalized
  - 15% HUS
  - 1% died (3 people – 2 adults, 1 child)
Role for Polymerase Chain Reaction Tests in the Diagnosis of STEC

- Detect \( stx1 \) and \( stx2 \) genes responsible for the production of Shiga toxin 1 and 2
- Used as a confirmatory tests by some PHLs
- Sensitivity of PCR assay on DNA extracted from direct stools is low
  - Do PCR on enrichment broth culture
- Currently not FDA approved
  - Establish performance characteristics
  - Include disclaimer that test is not cleared by FDA
- No PT available, exchange samples 2x/yr
Importance of Early Diagnosis of STEC Infection

- Aid in the differential diagnosis
  - Clinical presentation may mimic appendicitis, intussusception, IBD, C. difficile
- Avoid administration of antibiotics that may trigger release of toxin >> HUS
- Give early IV isotonic volume expansion
- Avoid secondary spread to family members
- Timely investigation to detect source and limit spread and secondary infections
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The findings and conclusions in this presentation represent those of the author and do not represent the official position of the CDC. Mention of specific diagnostics does not imply endorsement by the agency.