The Isolate Recovery Project: An Efficient CIDT-Positive Workflow

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Isolate Recovery Subcommittee

- Represented by State Public Health Labs (CO, IA, LA County (CA), MN, and TN), APHL, and CDC, and working to generate data to formulate recommendations for the efficient recovery of *Salmonella* and STEC (Shiga toxin-producing *E. coli*) from CIDT-positive specimens.
  - Media Study
  - Seeded Stool Study
Media Study Summary

- 75 STEC clinical isolates examined from 29 different serogroups
- 22 non-STEC isolates
- CHROM STEC and CT-SMAC suppressed commensal flora more than SMAC and WSB
- 36% STEC on CHROM STEC and 17% on CT-SMAC did not grow
  - Reduced growth for “top six” serogroups
- 72 *Salmonella* clinical isolates examined from 24 different serotypes
- 17 non-*Salmonella* isolates
- CHROM *Salmonella* and BS suppressed commensal flora more than HEK, SSI, and XLD
- 3% *Salmonella* did not grow on CHROM *Salmonella*
  - *Salmonella enterica* serotypes Muenchen and Concord

Creating a Homogenized Stool

Defrost at 4°C overnight

n grams

PBS

n grams

Stomacher

Centrifuge

All stools were tested by rtPCR for STEC- or *Salmonella*-specific targets and plated for suspicious growth prior to homogenization.
Creating a Homogenized Stool (cont.)

Strain A
- Low
- Broth inoculated & incubated overnight at 37°C
- High

Strain B
- Low
- Combine 3 parts Cary-Blair or GN Broth with 1 part stool
- High

Two vials from each group assayed for RNAseP and pathogen-specific target(s) to ensure homogeneity.
### STEC Seeded Stool Testing Variables

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Inoculum Size</th>
<th>Storage Temperature</th>
<th>Storage Media</th>
<th>Storage Time</th>
<th>Enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>O157</td>
<td>Low (10^7 CFU/mL)</td>
<td>4°C</td>
<td>Cary-Blair</td>
<td>1 day</td>
<td>GN Broth</td>
</tr>
<tr>
<td>O111</td>
<td>High (10^9 CFU/mL)</td>
<td>22°C</td>
<td>GN Broth</td>
<td>4 days</td>
<td>None</td>
</tr>
<tr>
<td>O104</td>
<td>Unseeded</td>
<td></td>
<td></td>
<td>7 days</td>
<td></td>
</tr>
<tr>
<td>Unseeded</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. CDC, Real-time PCR assay for Stx 1 and Stx 2, unpublished.
Day 1
- GN
- DNA extraction

Day 2
- 37°C overnight
- Chrom
- WSB-M
- CT-SMAC
- SMAC

Day 3
- 37°C overnight
- Re'am-Tim'e PCR Machine

Seeded:
- 5 colony picks selected from each plate

Unseeded:
- 10 colony picks selected from each plate

PCR also performed on DNA, enrichment, and SMAC sweep
Salmonella Seeded Stool Testing Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Newport</th>
<th>Oranienberg</th>
<th>Unseeded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculum Size</td>
<td>Low</td>
<td>High</td>
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<tr>
<td>Storage Temperature</td>
<td>4°C</td>
<td>22°C</td>
<td></td>
</tr>
<tr>
<td>Storage Media</td>
<td>Cary-Blair</td>
<td>GN Broth</td>
<td></td>
</tr>
<tr>
<td>Storage Time</td>
<td>1 day</td>
<td>4 days</td>
<td>7 days</td>
</tr>
<tr>
<td>Enrichments</td>
<td>Selenite</td>
<td>Tetrathionate</td>
<td>MSRV</td>
</tr>
</tbody>
</table>

Seeded: 3 colony picks selected from each plate for PCR
Unseeded: 6 colony picks selected from each plate

PCR also performed on DNA, enrichments, and HEK sweeps

Day 1  Day 2  Day 3  Day 4
# Reagents & PCR Conditions

- **Salmonella – invA**
  - 95°C for 3 min
  - 50 cycles (95°C for 15s, 60°C for 30s)
    - 10µL reactions

- **QiaAMP Fast DNA Stool Mini Kit**
- **Boil preps**
- **ABI Taqman Fast Universal PCR Mastermix (2X)**
- **Roche Lightcycler 96**

- **STEC – stx1 & stx2**
  - 96°C for 10 min
  - 40 cycles (95°C for 25s, 60°C for 25s)
    - 10µL reactions

- **QiaAMP Fast DNA Stool Mini Kit**
- **Boil preps**
- **ABI Taqman Environmental PCR Mastermix (2X)**
- **Roche Lightcycler 96**

2. CDC, Real-time PCR assay for Stx 1 and Stx 2, unpublished.
Cumulative Percent Positive by Colony Pick
STEC O104: CDC & IA
STEC O104 Low Inoculum
(5 colony picks)

Key:
- Negative
- Positive
- No Growth

CB – Stool stored in Cary Blair
GN – Stool stored in GN Broth
Direct – Stool plated directly
Enrich – Stool plated after enrichment

STEC O104 High Inoculum
(5 colony picks)

Key:
- Negative
- Positive
- No Growth

CDC

SMAC – Sorbitol MacConkey Agar
CT-SMAC – Cefixime-Tellurite SMAC
CHROM – CHROMagar STEC
WSB-M – Washed Sheep’s Blood w/ Mitomycin C
Cumulative Percent Positive by Colony Pick
STEC O111: CDC & IA
STECA O111 Low Inoculum
(5 colony picks)

STEC O111 High Inoculum
(5 colony picks)

**Key:**
- Negative
- Positive
- No Growth

<table>
<thead>
<tr>
<th>Storage Media &amp; Plating Conditions</th>
<th>Days of Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB, Direct</td>
<td></td>
</tr>
<tr>
<td>GN, Enrich</td>
<td></td>
</tr>
<tr>
<td>CB, Direct GN, Direct</td>
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<td></td>
</tr>
<tr>
<td>CB, Direct GN, Direct, Enrich</td>
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</tbody>
</table>

**Plating Media**
- SMAC – Sorbitol MacConkey Agar
- CT-SMAC – Cefixime-Tellurite SMAC
- CHROM – CHROMagar STEC
- WSB-M – Washed Sheep’s Blood with Mitomycin C

**Storage Temperature**
- 4°C
- 22°C

CDC
Conclusions

- O104 High and O111 Low & High recovered at ≥98% within 5 picks
- O104 Low recovered at 71% within 5 picks
- Lower Recovery:
  - 4°C storage temperature
  - GN transport media
  - Longer storage time
- O157 data (IA and MN) currently being analyzed
- *Salmonella* data (CDC and LA County) to be analyzed
- Goals:
  - Best screening point
  - Optimal pick number
  - Best shipping and storage conditions
  - Best plating media(s)
- Culminate in guidelines for laboratories
Our Collaborators:

- Department of Health and Human Services (HHS)
- Centers for Disease Control and Prevention (CDC)
- Association of Public Health Laboratories (APHL)
- Oak Ridge Institute for Science and Education (ORISE)
- CO Department of Public Health & Environment, Laboratory Services Division
- Los Angeles County Department of Public Health
- MN Department of Health, Public Health Laboratory
- TN Department of Health
- University of IA, State Hygienic Laboratory
For more information please contact Centers for Disease Control and Prevention

1600 Clifton Road NE, Atlanta, GA 30333
Telephone: 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348
Visit: www.cdc.gov | Contact CDC at: 1-800-CDC-INFO or www.cdc.gov/info

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.