Salmonella Isolate Recovery Project

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Enteric Diseases Laboratory Branch, CDC

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EDLB Project Contributors

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Jasmine Hensley

Brooke Aspinwall
Current Project

Replicated testing of the most promising conditions used in previous studies to identify an improved *Salmonella* isolate recovery workflow

STEC recovery testing beginning in spring 2019
Standard Stool Creation

60mL/donor → 300mL pooled stool

Salmonella Stock (Oranienburg or Newport)

Salmonella spike-in confirmed by plating and qPCR
Phase 1

Which transport media, holding temperature, and plating media best facilitate *Salmonella* recovery?
# Phase 1

## Experimental Design

<table>
<thead>
<tr>
<th>Variable</th>
<th>Oranienburg</th>
<th>Newport</th>
<th>Unspiked</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serotype</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Spike Level</strong></td>
<td>$10^4$</td>
<td>$10^2$</td>
<td>US</td>
</tr>
<tr>
<td><strong>Days Since Collection</strong></td>
<td>4</td>
<td>8</td>
<td>14</td>
</tr>
</tbody>
</table>

## Variables Tested

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cary-Blair</th>
<th>GN Broth</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transport Media</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Holding Temperature</strong></td>
<td>22°C</td>
<td>4°C</td>
</tr>
<tr>
<td><strong>Plating Media</strong></td>
<td>Hektoen</td>
<td>XLD</td>
</tr>
</tbody>
</table>


Sweeps

Darker is better

Picks

Mean Positive Sweeps

Mean Positive Picks

22°C

4°C

GN.XLD.Oranienburg.22-
CB.XLD.Oranienburg.22-
GN.HEK.Oranienburg.22-
CB.HEK.Oranienburg.22-
GN.XLD.Newport.22-
CB.XLD.Newport.22-
GN.HEK.Newport.22-
CB.HEK.Newport.22-
GN.XLD.Oranienburg.4-
CB.XLD.Oranienburg.4-
GN.HEK.Oranienburg.4-
CB.HEK.Oranienburg.4-
GN.XLD.Newport.4-
CB.XLD.Newport.4-
GN.HEK.Newport.4-
CB.HEK.Newport.4-
GN.XLD.Oranienburg.22-
CB.XLD.Oranienburg.22-
GN.HEK.Oranienburg.22-
CB.HEK.Oranienburg.22-
GN.XLD.Newport.22-
CB.XLD.Newport.22-
GN.HEK.Newport.22-
CB.HEK.Newport.22-
GN.XLD.Oranienburg.4-
CB.XLD.Oranienburg.4-
GN.HEK.Oranienburg.4-
CB.HEK.Oranienburg.4-
GN.XLD.Newport.4-
CB.XLD.Newport.4-
GN.HEK.Newport.4-
CB.HEK.Newport.4-
Phase 1 Outcomes

Cary-Blair is more widely used

Hektoen has a longer shelf-life

Recovery best when specimens transported/stored at 22°C
Phase 2 “Hot Truck”

Are heat spikes during transport detrimental to *Salmonella* recovery?
Phase 2

Experimental Design

Serotype
- Oranienburg
- Newport
- Unspiked

Spike Level
- $10^4$
- $10^2$
- US

Days Since Collection
- 4
- 7
- 14

Transport Media
- Cary-Blair

Plating Media
- Hektoen

Variables Tested

Holding Temperature
- 78°C
- 55°C
- 55°C
- 22°C
- 22°C
Darker is better

Picks

<table>
<thead>
<tr>
<th>Day</th>
<th>10^2</th>
<th>10^4</th>
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</thead>
<tbody>
<tr>
<td>4</td>
<td>4</td>
<td>7</td>
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<tr>
<td>7</td>
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Sweeps

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Mean Positive Picks

Oranienburg.ice.22
Newport.ice.22
Newport.no ice.22
Oranienburg.no ice.22
Oranienburg.ice.55
Newport.ice.55
Newport.no ice.55
Oranienburg.no ice.55
Newport.ice.78
Oranienburg.ice.78
Newport.no ice.78
Oranienburg.no ice.78

Mean Positive Sweeps

Oranienburg.ice.55
Newport.ice.55
Newport.no ice.55
Oranienburg.no ice.22
Newport.no ice.22
Oranienburg.ice.22
Newport.ice.22
Oranienburg.ice.22
Newport.ice.22
Phase 2 Outcomes

- No difference in recovery between specimens on ice vs 22°C
  - For Phase 3, hold samples at 22°C

- We recommend that during warmer months...

![Clinical Lab at 22°C](image1.png)

![SPHL Lab at 22°C](image2.png)
Phase 3

Which enrichments best facilitate *Salmonella* recovery?
Phase 3

Experimental Design

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<tbody>
<tr>
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<td>Hektoen</td>
<td>Cary-Blair</td>
<td>22°C</td>
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<tr>
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<td>$10^1$</td>
<td>MSRV</td>
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<td>US</td>
<td>TET37</td>
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</table>
100% Recovery!!
Salmonella Density

Commensal Density

Darker is better

Lighter is better
Phase 3 Outcomes

- 100% recovery across all enrichments
  - Tet37 and selenite perform similarly
- Selenite chosen based on:
  - Cost
  - Preparation difficulty/time
  - Commensal suppression
  - Shelf-life
The Golden Path

Specimen in Cary-Blair

Clinical Lab

22°C

SPHL Lab

22°C

Recover off SEL -> HEK if possible

Recover off HEK, if possible

Confirmatory test
Our Collaborators:

- Department of Health and Human Services (HHS)
- Centers for Disease Control and Prevention (CDC)
  - EDLB: Michele Parsons, Efrain Ribot, Peter Gerner-Smidt, Cheryl Bopp, Michelle Sanders, Samantha Olson, and Christine VanTubbergen
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- Wireform, US
- Strokeicon
- Wilson Joseph
- Brand Mania

- Nikita Kozin
- Shastry
Thank you!

For more information, contact CDC
1-800-CDC-INFO (232-4636)

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.