

The Isolate Recovery Project: An Efficient CIDT-Positive Workflow

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PulseNet Session IV: From Isolate Recovery to the Future of CIDTs
Friday November 20th, 2015

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

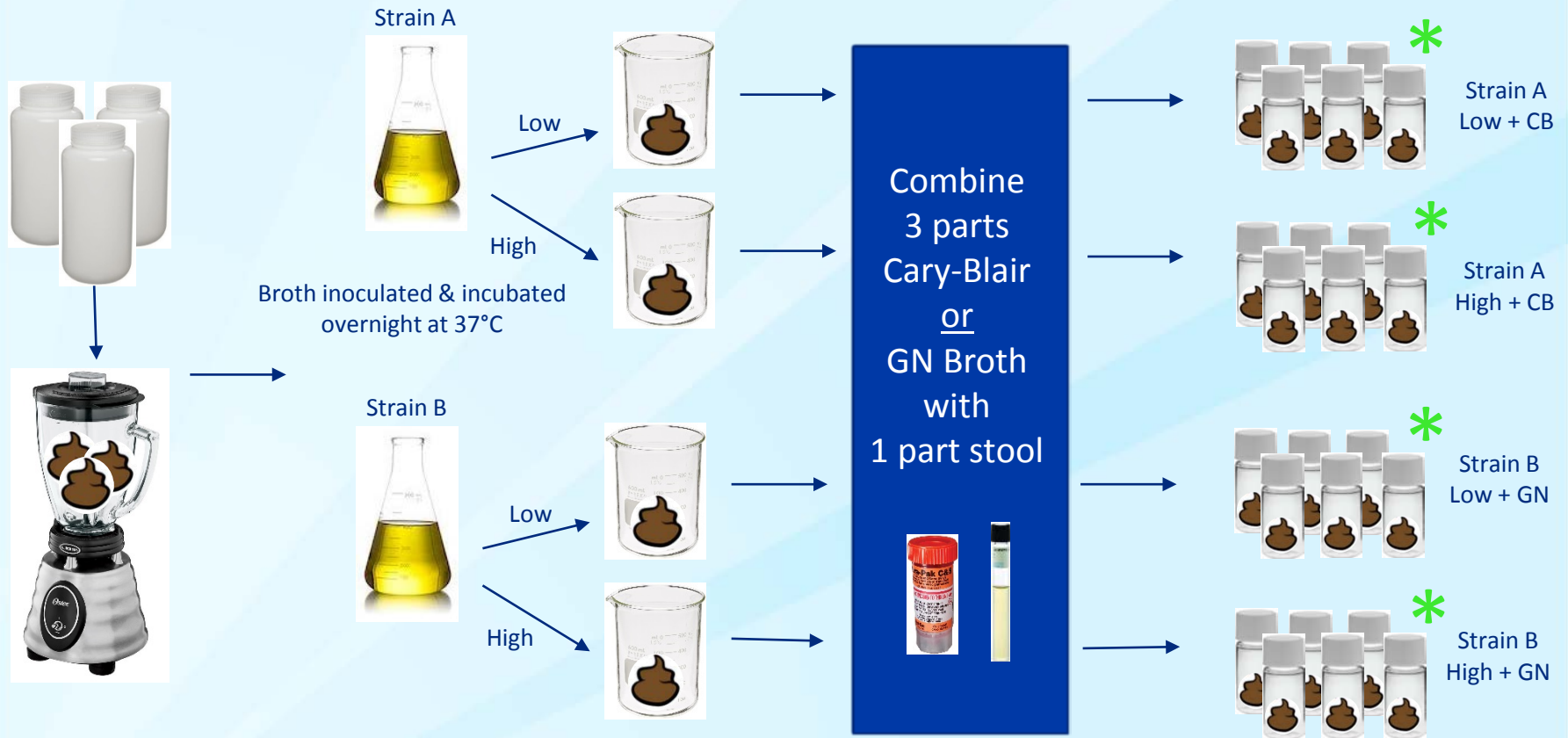
- **75 STEC clinical isolates examined from 29 different serogroups**
- **22 non-STECS isolates**
- **CHROM STEC and CT-SMAC suppressed commensal flora more than SMAC and WSB**
- **36% STEC on CHROM STEC and 17% on CT-SMAC did not grow**
 - Reduced growth for “top six” serogroups
- **72 *Salmonella* 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



* 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.)



Two vials from each group assayed for RNaseP and pathogen-specific target(s) to ensure homogeneity.

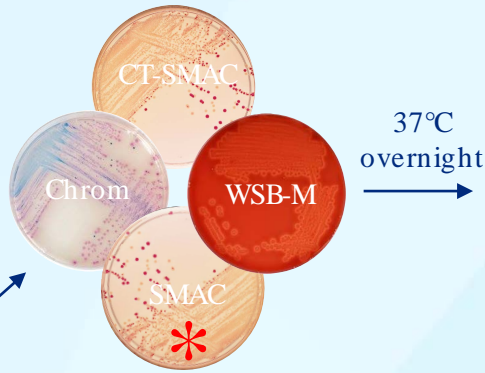
STEC Seeded Stool Testing Variables

Serogroup	Inoculum Size	Storage Temperature	Storage Media	Storage Time	Enrichment
<ul style="list-style-type: none">• O157• O111• O104• Unseeded	<ul style="list-style-type: none">• Low (10⁷ CFU/mL)• High (10⁹ CFU/mL)• Unseeded	<ul style="list-style-type: none">• 4°C• 22°C	<ul style="list-style-type: none">• Cary-Blair• GN Broth	<ul style="list-style-type: none">• 1 day• 4 days• 7 days	<ul style="list-style-type: none">• GN Broth• None

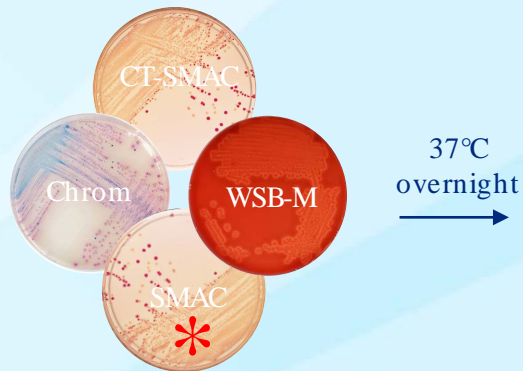
1. Jinneman, K.C., Yoshitomi, K.J., & S. J. Weagant. (2003). "Multiplex Real-time PCR Method to Identify Shiga Toxins, Stx 1, Stx 2, and *E. coli* O157:H7 Serogroup." *Applied and Environmental Microbiology*. 69 (10), 6327-6333.
2. CDC, Real-time PCR assay for Stx 1 and Stx 2, unpublished.



Real-Time PCR Machine



Real-Time PCR Machine



DNA extraction *



Day 1

Day 2

Day 3

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

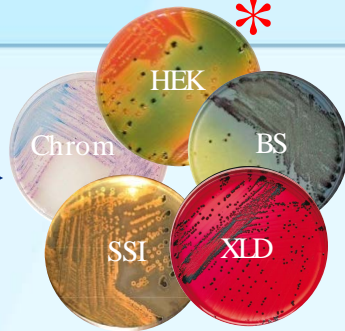
Serotype	Inoculum Size	Storage Temperature	Storage Media	Storage Time	Enrichments
<ul style="list-style-type: none">• Newport• Oranienberg• Unseeded	<ul style="list-style-type: none">• Low (10⁷ CFU/mL)• High (10⁹ CFU/mL)• Unseeded	<ul style="list-style-type: none">• 4°C• 22°C	<ul style="list-style-type: none">• Cary-Blair• GN Broth	<ul style="list-style-type: none">• 1 day• 4 days• 7 days	<ul style="list-style-type: none">• Selenite• Tetrathionate• MSRV• None

1. Timme, R. E., Pettengill, J., et al. (2013). "Phylogenetic Diversity of the Enteric Pathogen *Salmonella enterica* subsp. *enterica* Inferred from Genome-Wide Reference-Free SNP Characters." *Genome Biology and Evolution*, 5(11), 2109-2123.

DNA extraction



37°C overnight



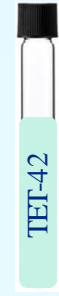
37°C overnight



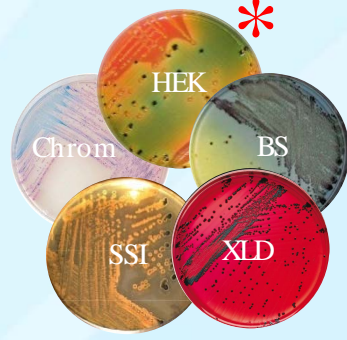
Real-Time PCR Machine



37°C overnight



42°C overnight



37°C overnight



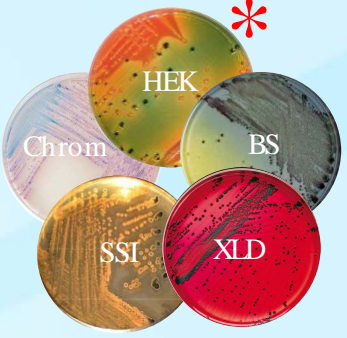
Real-Time PCR Machine



37°C overnight



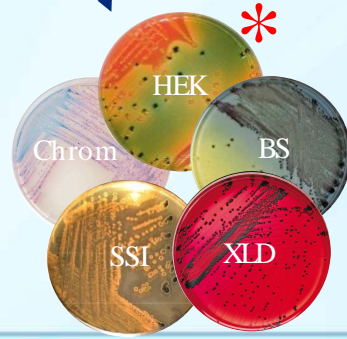
42°C overnight



37°C overnight



Real-Time PCR Machine



37°C overnight



Real-Time PCR Machine

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

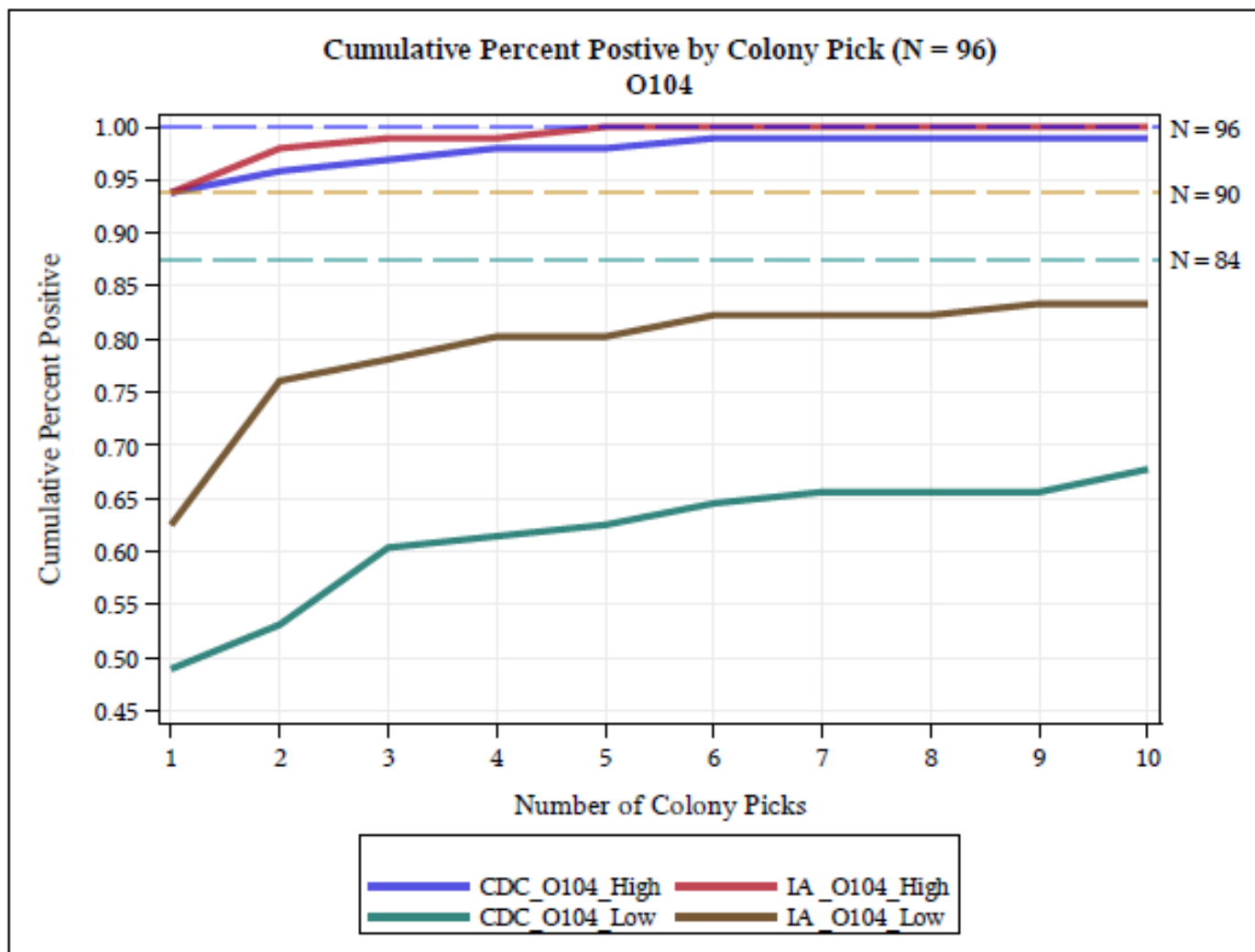
Reagents & PCR Conditions

- ***Salmonella* – *invA*¹**
 - 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*²**
 - 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**

1. Cheng, C.M., Lin, W., *et al.* (2008). Rapid Detection of *Salmonella* in Foods Using Real-Time PCR. *Journal of Food Protection*, 71(12), 2436-2441.

2. CDC, Real-time PCR assay for Stx 1 and Stx 2, unpublished.

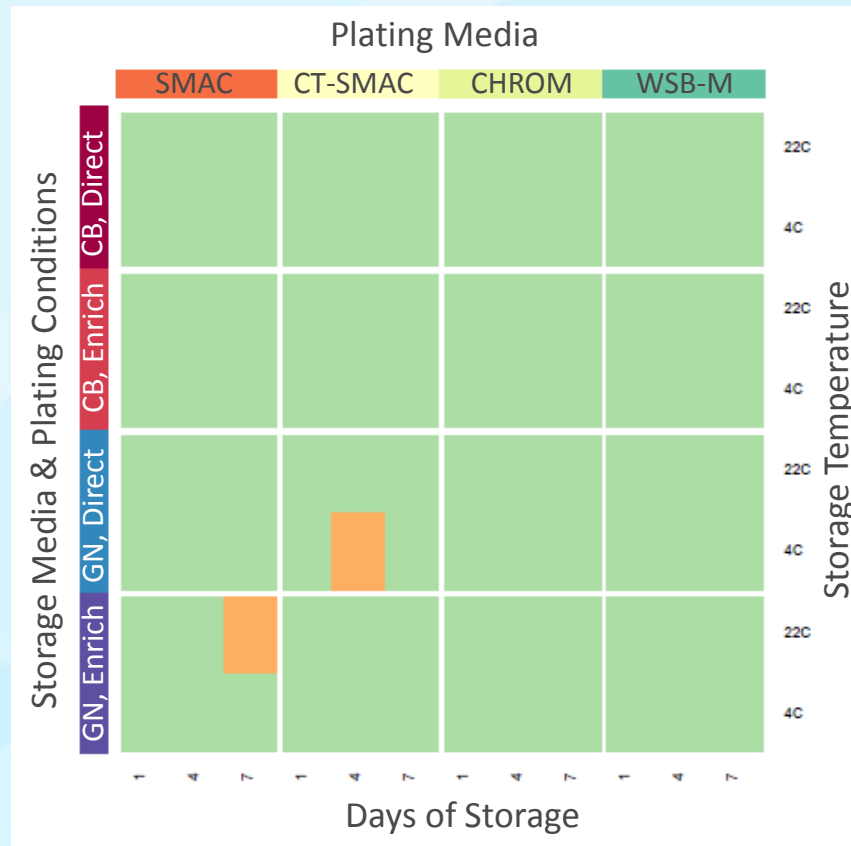
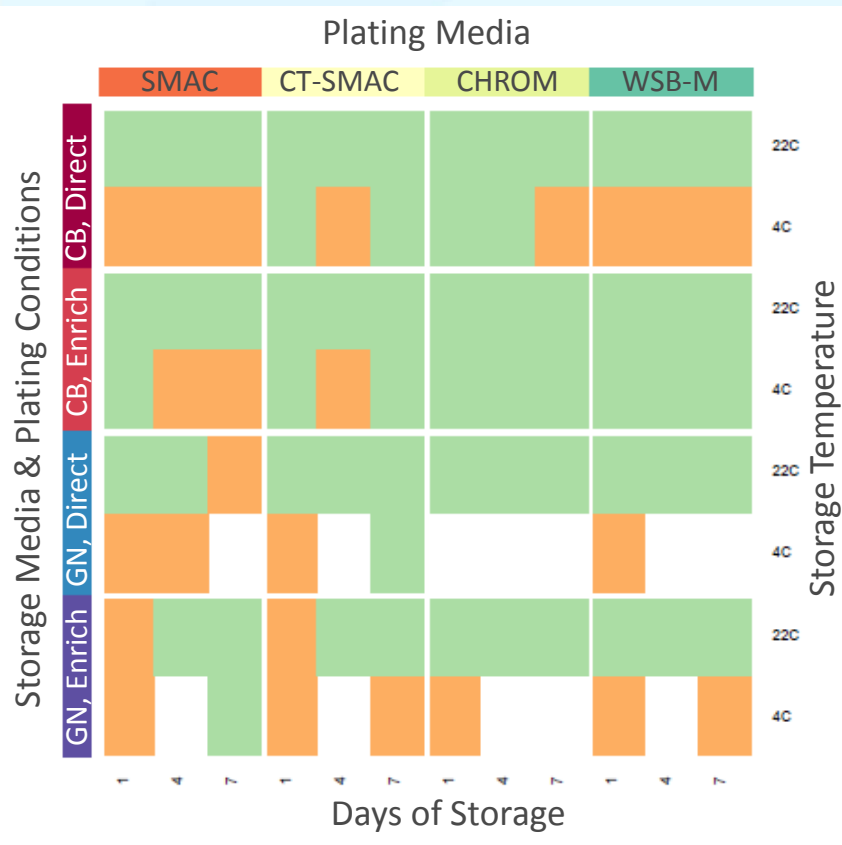
Cumulative Percent Positive by Colony Pick STEC O104: CDC & IA



STE C O104 Low Inoculum (5 colony picks)

CDC

STE C O104 High Inoculum (5 colony picks)



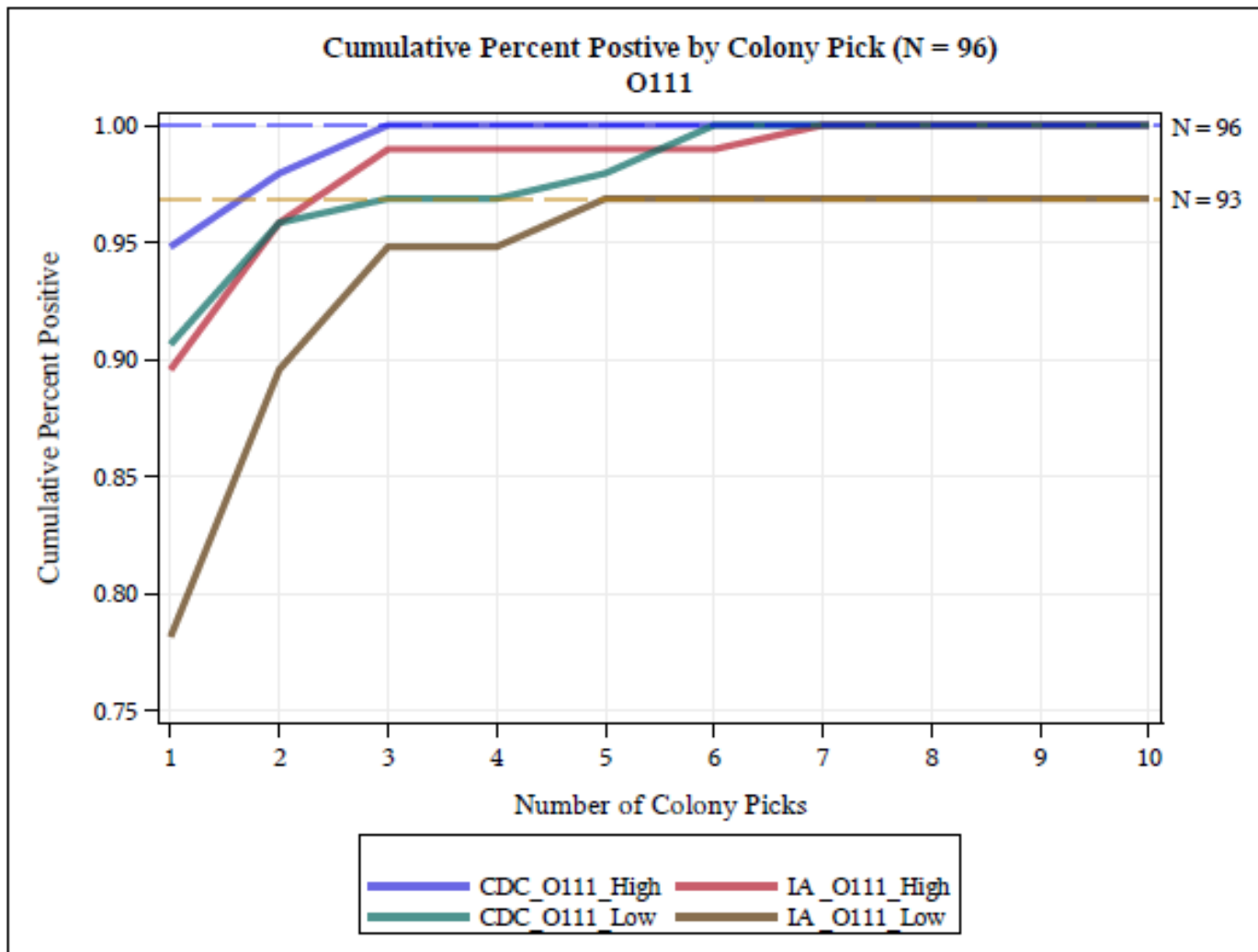
Key:

- - 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

SMAC – Sorbitol MacConkey Agar
 CT-SMAC – Cefixime-Tellurite SMAC
 CHROM – CHROMagar STEC
 WSB-M – Washed Sheep's Blood w/ Mitomycin C

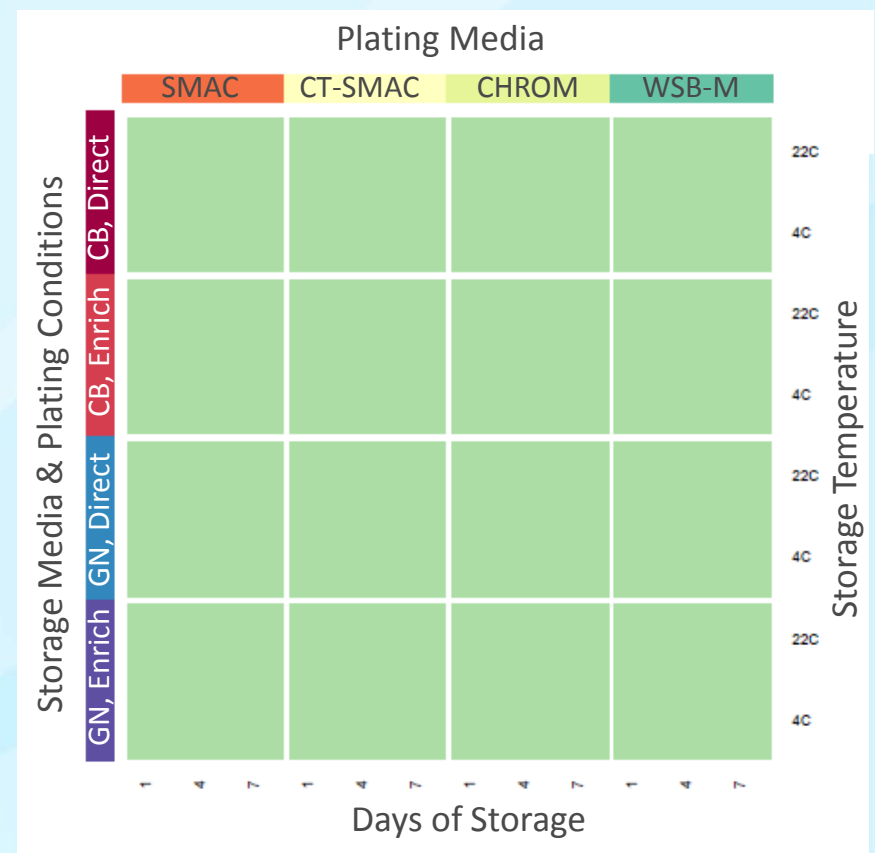
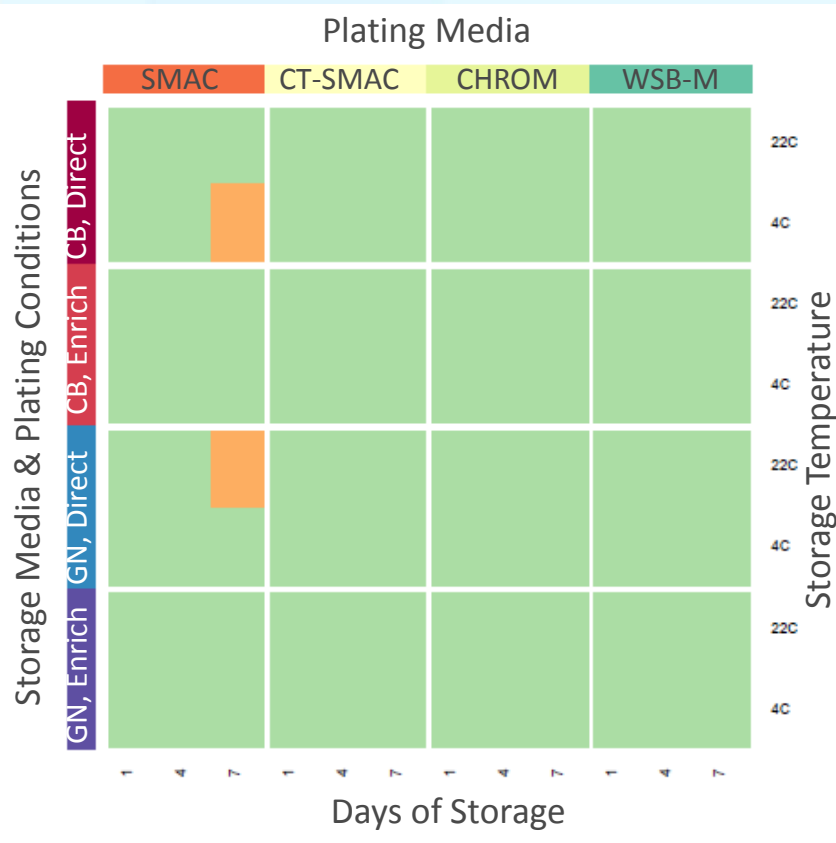
Cumulative Percent Positive by Colony Pick STEC O111: CDC & IA



STE C O111 Low Inoculum (5 colony picks)

CDC

STE C O111 High 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

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

Conclusions

- **O104 High and O111 Low & High recovered at $\geq 98\%$ 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.

