Optimizing Rapid Test Methods for Shigatoxin Producing E. coli (STEC) on Fresh Produce and in Ag-Environments

Webinar Presented 18 August 2014
Optimizing Rapid Test Methods for Shigatoxin Producing E. Coli (STEC) on Fresh Produce and in Ag-Environments

AGENDA

1. Introduction  Bonnie Fernandez-Fenaroli – CPS
2. Webinar Mechanics  Hank Giclas – WG
3. Industry Perspective  Mary Zischke - CLGRB
4. Plain Language Overview  Trevor Suslow – UC Davis
5. Resolving Decision-making Uncertainties  
   a. STEC diversity on produce  Evan Chaney - ROKA  
   b. Actionable/non-actionable STEC  Sam Myoda – IEH
6. Q&A  Hank
7. Closing Statement/Next Steps  Bonnie
Mary Zischke

California Leafy Greens Research Program
Industry Perspective

- Since 2006 many changes implemented to safeguard public health
  - Comes at a significant cost
- Major component of many food safety programs: raw product testing
  - Range of sampling programs
  - Range of sampling protocols
  - Quick turnaround and accuracy both crucial
Optimizing Rapid Test Methods for Shigatoxin Producing E. coli (STEC) on Fresh Produce and in Ag-Environments

Rapid Testing

- Positive results are rare events
- Lack of clarity about what the test results mean and what action to take
  - What are they really testing for?
  - What does “presumptive positive”, “can’t rule out”, etc. mean?
  - Are positive findings clinically relevant?
- Balancing Act between ensuring public safety and ensuring that safe product remains in the supply chain
Rapid Testing

- Positive results – next steps
  - Culture confirm or other follow-up tests
  - Company policies vary
    - Finding the balance: do the right thing, avoid being overly cautious
  - Regulatory Guidance not clear
  - Creates doubts about food safety programs’ effectiveness
Optimizing Rapid Test Methods for Shigatoxin Producing E. coli (STEC) on Fresh Produce and in Ag-Environments

Crop Loss has important impacts

- Resources used to produce the crop lost
- Creates a shortage in the supply chain
  - Not a lot of flex in the system
    - Harvest next field early
    - Spot Purchases
    - Difficulty in filling orders
- Growers’ Costs Increase
Industry asking for clarification

- Address the accuracy issue
- Guidance on what a testing program can and can’t do to ensure the safety of our products
- Eliminate unnecessary crop loss
Trevor Suslow

University of California at Davis
Optimizing Rapid Test Methods for Shigatoxin Producing *E. coli* (STEC) on Fresh Produce and in Ag-Environments

- **Technical level**
  - Responding to requests; Simple descriptions to start
  - Moving stepwise to slightly more detail
  - Understanding test methods and Actionable Decision Points

- **Future events & meeting products**
  - Issue brief on STEC/EHEC; online access
  - Technically-oriented event – webinar or workshop
Optimizing Rapid Test Methods for Shigatoxin Producing *E. coli* (STEC) on Fresh Produce and in Ag-Environments

- **To get started efficiently**
  - We need to assume that STEC and EHEC are familiar terms
  - They are confusing; definitions and examples will unfold
- **Goal for today’s webinar**
  - Answer your questions about STEC Test methods
  - Answer your questions about STEC Decision Tree
Optimizing Rapid Test Methods for Shigatoxin Producing \textit{E. coli} (STEC) on Fresh Produce and in Ag-Environments

• Disclaimer – Mention of specific test developers or products is for informational purposes only and does not necessarily imply endorsement of these methods nor the inadequacy of options not presented or discussed.
Preharvest testing for ‘EHEC’ has resulted in many cases of whole field destruction that may have been due to detection of virulence markers in STEC from multiple cells, including related non-E. coli strains, in the enrichment culture.
Suppliers appealed to research science and public health experts to distill the complexity of STEC diversity and testing into simpler knowledge-based learning’s and standards.

Discing a lettuce field ‘flagged’ as being contaminated with EHEC in a preharvest assessment.
Unnecessary crop destruction works against company and industry initiatives in agricultural sustainability

Field 4 days after preharvest sample was ‘positive’ for STEC
Detection of STEC is Very Relevant to Diverse Crop, Soil, and Water Protection Practices

Ex: Windbreaks protect soil, water, encourage beneficial’s, reduce dust aerosol transfer, and reduce injury to crops, especially tender greens
Objective criteria are needed for prioritizing potentially conflicting initiatives at the farm and regional landscape level.

Habitat for birds, reptiles and amphibians have been identified as a possible source of STEC and EHEC. Only reacting to types that pose an established public health risk is desired.
EHEC/STEC in Produce Colloquium
May 30-31, 2013

Hosted by UCGAPs & CPS
Jointly Sponsored by
PMA, WG, CLGRB, CPS
Colloquium Participants (FDA, CDC, ARS, CPS)

- Joseph M Bosilevac (Mick), Ph.D., Research Microbiologist, Agricultural Research Service, USDA
- Michael Cooley (Mike), Ph.D., Research Geneticist, Produce Safety and Microbiology Research, ARS/USDA
- Peter Feng, Ph.D., Research Microbiologist, STEC/EHEC Specialist, U.S. Food and Drug Administration
- Bonnie Fernandez-Fenaroli, Executive Director, Center for Produce Safety
- Pina M Fratamico, Ph.D., Research Leader, Molecular Characterization of Foodborne Pathogens, ARS/USDA
- Henry (Hank) Giclas, Senior Vice President, Science, Technology & Strategic Planning, Western Growers
- Vivek Kapur, BVSc, Ph.D., Professor, Dept. Veterinary and Biomedical Sciences, Penn State University
- Robert Mandrell, Ph.D., Research Leader (Retired), Produce Safety/Microbiology Research Unit. ARS/USDA
- Courtney Parker, Ph.D., Vice President of Food Safety and Quality, Chiquita Brands International, Inc.
- Nancy Strockbine, Ph.D., Chief, Escherichia and Shigella Reference Unit, Enteric Diseases Laboratory Branch Division of Foodborne, Waterborne, and Environmental Diseases, OID/NCEZID, Centers for Disease Control and Prevention
- Trevor V. Suslow, Ph.D., Extension Research Specialist, University of California, Davis
- Robert (Bob) J. Whitaker, Ph.D. Chief Science & Technology Officer, PMA
Recommended Products from Colloquium

• Regional industry meetings and other outreach -
  – Webinar series
• Issue Brief summarizing colloquium notes -
• Detailed industry-technical note – in progress
  – Delayed by farm-level discoveries
• Targeted research to resolve confusion – in progress
Examples of Questions Received for Webinar

- Our buyer requires that we test for E. coli O157 and EHEC; Is an STEC test good enough?

- I am really lost. Would you please make this something a grower can follow. Is there an easy way to get why I can’t have a Yes/No test on preharvest samples?
**What you need to know**

- *E. coli* O157:H7 is an EHEC
- All EHEC are also STEC
- All STEC have some traits that are of public health concern
- All STEC are in the general Coliform group
- Coliforms are **not** useful indicators of EHEC on produce
*E. coli* cause human gastrointestinal illness and serious or lethal infections

- **Shiga toxin-producing (STEC)**
  - a highly diverse and variable group of non-pathogens, opportunists, and highly serious pathogens

- **Enterohemorrhagic (EHEC) - diversity is catalogued by serotype**
  - *E. coli* O157 serogroup
  - Non-O157 serogroups

Both groups can cause serious and deadly infections
The diversity of STEC makes decisions based on combinations of detectable markers challenging?

- Markers must be in a single cell to be a pathogen
- This essential information not always known in test
- Detected markers known to occur in non-pathogens
- Some markers lost in environmental adaptation
- Traits and markers lost in lab manipulation
  - Stability is different among STEC types
Examples of Questions Received for Webinar

• Our buyer requires that we test for E. coli O157 and EHEC; Is an STEC test good enough?

• I am really lost. Would you please make this something a grower can follow. Is there an easy way to get why I can’t have a Yes/No test on preharvest samples?
Taking a long-shot at a simple analogy

• Think of STEC like an MLB team
External Markers of SF Giants take multiple forms

Evolutionary Early Form
Individual Variation in Markers we can recognize and therefore predict Traits
Individuals have external markers that make identification easier.
Members of the same group exchange critical information

Under special conditions the group will auto-aggregate
If you only have tools to see one or two markers you could make a mistake identifying the real player from non-player
If you only have tools to see one or two markers you could make a mistake identifying the real player from non-player.
Individuals can acquire new traits or external markers.....becoming more aggressive
The surrounding adjacent area is full of non-players with ‘markers’
Taking a long-shot at a simple analogy

• Think of STEC like an MLB team

• Now comes the tough part
Stx producing bacteria – Which are a health risk?

**STEC**

Shiga-toxin producing *E. coli*

~300 serotypes

*testing Stx or stx is generally easy*

- Stx₁, Stx₁c, Stx₁d
- Stx₂, Stx₂c, Stx₂d, Stx₂e, Stx₂f, Stx₂g

**EHEC**

O₁57:H7

EPEC

**eae**

α, β, γ, ~30

- EHEC
- EPEC

EHEC O₁₁₃:H₂₁

O₉₁:H₂₁

Cardinal Trait Approach
Stx + binding factor = Severe disease

Stx producing bacteria – Which are a health risk?

**STEC**

shiga-toxin producing *E. coli*

~300 serotypes

*testing Stx or stx is easy but not sufficient*

**EHEC**

*α,β,γ, ~30*

**EPEC**

**Shigella spp.**

**ETEC**

**EAEC**

**O104:H4**

**O157:H7**

**EHEC**

**O113:H21**

**O91:H21**

Cardinal Trait Approach

Stx + binding factor = Severe disease
Stx producing bacteria – Which are a health risk?

- **E. cloacae**
- **C. freundii**
- **Citrobacter**
- **Klebsiella**

**STEC**

Testing for stx and eae alone is not sufficient to know with certainty.

- **EHEC**
  - eae \(\alpha, \beta, \gamma, \sim 30\)

**EPEC**

- **O157:H7**
- **EHEC**

**Cardinal Trait Approach**

Stx + binding factor = Severe disease
Stx producing bacteria – Which are a health risk?

E. cloacae
C. freundii
Shigella spp.
E. coli

STEC
shiga-toxin producing E. coli
~300 serotypes

EHEC
α, β, γ, ~30

EPEC

It’s a bit of a mess!

E. cloacae
C. freundii
Shigella spp.

E. coli

STEC
shiga-toxin producing E. coli
~300 serotypes

EHEC
α, β, γ, ~30

EPEC

Cardinal Trait Approach
Stx + binding factor = Severe disease

Stx1, Stx1c, Stx1d
Stx2, Stx2c, Stx2d, Stx2e, Stx2f, Stx2g

EAEC
O104:H4

EAEC
O113:H21
O91:H21

O157:H7
EHEC

EAEC
O104:H4

EHEC

EAEC
O113:H21
O91:H21

Citrobacter
Klebsiella

Cardinal Trait Approach
Stx + binding factor = Severe disease
Test Method Validation & ‘Certification’ Simplified

- AOAC RI – Association of Official Analytical Chemists
  - Independent, 3rd-party non-government administrator of standards and conformity assessments of test methods
  - Test reliability and reproducibility; standardized criteria
  - Several ‘certification’ options

- AOAC RI PTM – Performance Tested Method
  - Widely recognized as authoritative method certification program for proprietary methods
  - Limitations become evident with “real-world” applications and broad use
Test Method Validation & ‘Certification’ Simplified

• Service labs must use developer Certified Test Method or PTM specifications
  – Their protection in Actionable Decisions
  – Followed certified protocols for sample enrichment
    • Sample Mass, Temperature, Time, Processing, Detection

• May vary by external or internal validation studies
  – Should go through the AOAC or equivalent process
  – Too often the modified methods are incompletely assessed
Immunological Testing

• Like a ‘pregnancy’ quick test
• Examples to detect pathogens
  – VIDAS (bioMerieux)
  – EIA (BioControl)
  – Reveal (Neogen)
  – Rapidchek (SDI)
  – others
• Demonstrated to be less sensitive in primary tests
• Useful in secondary or culture-dependent tests
• Similar tests for Shigatoxin protein
  – Can be diagnostic for toxin production
  – Some sensitivity concerns – false negative
ImmunoMagnetic Separation (IMS) May Be a Critical Step In STEC Detection and Recovery

Magnet

IMS Microbead

Tangential Flow

Mixed Bacteria in Broth Growth Media
ImmunoMagnetic Separation (IMS) May Be a Critical Step In STEC Detection and Recovery
ImmunoMagnetic Separation (IMS) May Be a Critical Step In STEC Detection and Recovery

Mixed Bacteria in Broth Growth Media

Captured Target Cells (like Eco157)

Tangential Flow

Re-circulating flow or Mixing

Magnet

IMS Microbead
ImmunoMagnetic Separation (IMS) May Be a Critical Step In STEC Detection and Recovery

- Target cells may be lost in washing
- Non-target binding blocks sites
- Non-target cells greatly complicate culture recovery
- Typically attempt culture confirmation with and without IMS
- Can take 5-8 days on multiple media
- Not practical for timely decisions
**Isolates obtained from field ‘flagged’ as EHEC positive in preharvest testing**

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Examples of Detection of STEC virulence genes in Preharvest Tests and Rapid Response isolates

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<th>eae</th>
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<th>stx2</th>
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<th>ehxA</th>
<th>saa</th>
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<th>ehxA</th>
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*Strains from product testing  
**Strains from Jay-Russell WCFS + Suslow Lab collaborative Rapid Response  
§ Known *E. coli* O157:H7
FDA and USDA are looking hard at commodity-based risk attribution

The prevalence of Shiga toxin subtypes and selected other virulence factors among Shiga toxigenic *Escherichia coli* strains isolated from fresh produce

PETER FENG and SHANKER REDDY

*Appl. Environ. Microbiol.*

### FDA Produce STEC – 2002 - 2012

<table>
<thead>
<tr>
<th>Commodity</th>
<th>STEC</th>
<th>O157:H7</th>
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<tr>
<td>Cantaloupe</td>
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<tr>
<td>Celery</td>
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<tr>
<td>Cilantro</td>
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<tr>
<td>Hot peppers</td>
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<tr>
<td><strong>Lettuce</strong></td>
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<tr>
<td>Parsley</td>
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<tr>
<td>Spinach</td>
<td>70</td>
<td>3</td>
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<tr>
<td>Alfalfa sprouts</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Tomatoes</td>
<td>4</td>
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<tr>
<td><strong>Total</strong></td>
<td>133</td>
<td>5</td>
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</table>

- Over 50% of STEC from Spinach
- Not all clinically relevant
- Complex isolate variants to resolve
- Recall not always recommended by FDA
- Decisions already involve clinical relevance factors
Changing Approach to FDA Determination of Case by Case STEC Risk: 3rd-Gen MicroArray
Next Up

• Further background on the challenges of STEC diversity and lot acceptance decisions

• Why we held off presenting a webinar on the issues discussed in Colloquium
Moving on to Answer the Questions

- Our buyer requires that we test for E. coli O157 and EHEC; Is an STEC test good enough?

- Why I can’t have a Yes/No STEC?
Evan Chaney

Roka Bioscience
Pathogenic *E. coli*
Are STEC the same as EHEC?

Both have stx and both may have eae genes
EHEC contain stx, eae, and ehxA
Why are EHEC important?

EHEC (enterohemorrhagic E. coli) is a CLINICAL term that describes shiga toxin producing E. coli associated with severe disease such as hemorrhagic colitis and hemolytic uremic syndrome (like E. coli O157:H7).

Studies have shown a strong correlation between disease and EHEC associated genes – stx, eae, and ehxA.
Continuing Evolution of Methods

More specific and diagnostic targets are needed to detect the range of clinically relevant STEC/EHEC.

- **stx1/stx2 alone is not definitive**
  - Unstable, especially in STEC
  - Mobile
  - Can be lost in the field or during the testing process
  - Other bacteria contain stx genes

- **Cannot discriminate “co-presence”**
  - *eae* from one cell and *stx* from another cell could cause false positive results
  - No method can easily decipher
  - More markers work

- **More than “Big 6”**
  - STEC/EHEC cover hundreds of serogroups with hundreds of strains,
  - Some not previously well recognized
Shiga toxin Pitfalls:

Testing for Shigatoxin
- PCR - Looking for the stx gene
- Lateral Flow - Looking for the STX protein (expression of the gene)

Shigatoxin Loss
- Shiga toxin is on a mobile element (prophage)
- Can be induced, excised and lost in the field
- Can also be lost in culture or enrichment
- stx is less stable in non-O157 STECs than O157 STECs

Shiga toxin Specificity
- Other organisms such as Shigella, Citrobacter, and Enterococcus can carry shiga toxin genes
- May be detecting prophage (virus-like) itself

Shiga toxin loss makes it ineffective as a primary or sole screen for EHEC
Roka Approach

Roka uses the “EHEC associated gene target “ecf1” as a surrogate marker for the presence of stx, eae, and ehxA

ecf1= stx + eae + ehxA

ecf1 is highly correlated to EHEC and therefore clinical outcome

More Specific in identifying pathogenic bacteria that contain the genes that cause severe human illness
What is a Surrogate Marker

A biological marker intended to substitute for a clinical endpoint

- Elevated cholesterol is a surrogate marker for heart disease risk
- Therefore, Pharmaceuticals that lower cholesterol are approved as impacting heart disease without showing any impact of death

ecf= EHEC
Roka STEC/EHEC Approach in Food Industry

- AOAC Method Validation included 50 Inclusive Strains & 30 Exclusive strains
- Roka has evaluated over 500 characterized isolates
Roka STEC/EHEC Approach in Food Industry

AOAC Method Validation included 50 Inclusive Strains & 30 Exclusive strains

<table>
<thead>
<tr>
<th>Expected Positive</th>
<th>Strain/Organism</th>
<th>stx1</th>
<th>stx2</th>
<th>eae</th>
<th>Atlas result</th>
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<td></td>
<td>E. Coli 055:H7</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>USMARC_GB_STEC_001</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>E. coli O1:H11</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Roka has evaluated over **500** characterized isolates
What about Produce?

- STEC/EHEC diversity presents challenges to all test methods

- Diversity is becoming better understood as molecular methods are utilized more frequently

- Roka is partnering with academic & industry partners, to better understand unique STEC diversity in produce
**Roka Bioscience STEC/EHEC Approach**

*ecf* as a primary screen and a *stx* follow on enables higher confidence in lot acceptance decisions.

- Atlas STEC Screen
  - Positive
  - Negative
- stx Reflex test
  - Positive
  - Negative
- STEC/EHEC Positive
- STEC/EHEC Negative

Approximately 15-17 hours Total to molecular confirmation
Method Comparison

- = EHEC stx+
- = stx+ non E coli / non-EHEC
- = EHEC stx-
- = background flora

Leafy Green Samples → stx screen with PCR → culture confirmation

3-5 days

hours

culture confirmation

Leafy Green Samples → ecf screen with Atlas → stx reflex
Benefits of Roka Approach

- Single surrogate primary screen
  - Highly linked to clinically relevant *E. coli*
- Eliminate co-presence false positive risk
  - Single target primary screen ensures genes are in a single cell eliminating false positives
- Higher confidence in lot acceptance
  - Two target system pairing clinical target with a diagnostic target provides rapid molecular confirmation
- Higher sensitivity reduces false negative risk
  - Roka assay has a lower LOD minimizing potential False negative risk
- Validation
  - AOAC approved methodology
Sam Myoda

IEH Laboratories and Consulting
Which *E. coli*?

How do I know?

Samuel P Myoda, PhD

sam@iehinc.com

831.261.0076
Pathogenic Groups

- All *E. coli*
- EHEC
- STEC
- Big Six
- EPEC
- EIEC
- ETEC
- EHEC
### EHEC (Enterohemorrhagic *E. coli*)

**TABLE 1.** *Verotoxigenic Escherichia coli* serotypes associated with *bloody diarrhea or hemolytic uremic syndrome*<sup>a</sup>

| O4:H10; H−  | O50:H7; H− | O113:H2; H21 |                     |
| O6:H2; H−   |                     | O115:H10 | O157:H7; H− |                     |
| O26:H11; H− |                     | O119:H6 | O168:H− |                     |
| O38:H21     | O98:H− | O121:H19 | O?:H2; H7; H19; H21 |
| O45:H2      | O103:H2 | O125:H− |                     |
| O46:H31     | O104:H2; H21 | O128:H2; H25; H− |
|             | O105:H18 |                     |
|             | O111:H2; H8; H− |

# USDA MDP Virulent *E. coli* (2004-09)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinach</td>
<td>4,406</td>
<td>24</td>
<td>0.54%</td>
</tr>
<tr>
<td>Cilantro</td>
<td>2,277</td>
<td>9</td>
<td>0.40%</td>
</tr>
<tr>
<td>Parsley</td>
<td>1,707</td>
<td>5</td>
<td>0.29%</td>
</tr>
<tr>
<td>Lettuce</td>
<td>10,542</td>
<td>13</td>
<td>0.12%</td>
</tr>
<tr>
<td>Onion</td>
<td>2,078</td>
<td>2</td>
<td>0.10%</td>
</tr>
<tr>
<td>Hot Peppers</td>
<td>1,745</td>
<td>1</td>
<td>0.06%</td>
</tr>
<tr>
<td>Green Onions</td>
<td>7,191</td>
<td>3</td>
<td>0.04%</td>
</tr>
<tr>
<td>Sprouts (Alfalfa)</td>
<td>6,947</td>
<td>2</td>
<td>0.03%</td>
</tr>
<tr>
<td>Cantaloupe</td>
<td>9,985</td>
<td>2</td>
<td>0.02%</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>13,337</td>
<td>0</td>
<td>0.00%</td>
</tr>
</tbody>
</table>
CDC National Enteric Disease Surveillance

- Accounting for under-diagnosis and under-reporting, an estimated 96,534 *E. coli* O157 EHEC and 168,698 non-O157 EHEC (265,232 TOTAL) infections occur each year with more than 3,600 hospitalizations and 30 deaths

- 63.6% of EHEC infections are non-O157
Testing for *E. coli* O157 & non-O157 EHECs

- Needs to be *E. coli*
- Produces Shiga toxin (stx)
- Has attachment/effacement gene (e.g. eae)
  - will attach to walls of intestine/colon, damage cells and allow shiga toxin to rapidly be absorbed

Note: any *E. coli* with attachment/effacement can gain ability to produce shiga toxin and become an EHEC e.g. *E. coli* O104
Polymerase Chain Reaction (PCR) to detect EHECs

- Has to have:
  - uidA -> E. coli
  - stx -> shiga toxin
  - eae, subtilase or aagR -> attachment/effacement

- Note: FDA will act on all eae & stx

*E. coli*, others case by case
Polymerase Chain Reaction (PCR) to detect EHECs

- Need to make sure:
  - All signals coming from one organism
    - that is, the signal in not a composite:

[Diagram showing]

- Bacteria A: eae
- Bacteria B: uidA
- Bacteria C: stx

Looks like an EHEC but it is not
Two Step PCR Approach

Is designed to eliminate the chance of both false positive and false negatives.

The initial screen casts a “wide” net to make sure nothing is missed.
Detects required genes

Molecular Confirmation/Secondary Screening (4-6 additional reactions with and w/out immunomagnetic bead separation) ensure there are no false positives.
Rules out other organisms and composite signals
IEH Kits for *E. coli* O157 Detection

IEH Laboratories
Salmonella, EHEC, *E. coli* O157

- USDA/FSIS
  - Regulatory
  - School Lunch
- FDA
  - Regulatory, DWPE
  - Routine
- DAFF (Australia)
- Health Canada
The two step approach

1. Raw material, finished product or grower input sample
2. Screening Test
   - E. coli O157:H7
   - Non-O157 EHEC
   - Salmonella
   - MDR Salmonella

   Test results NEGATIVE
   - Sample free of pathogens
   - One or more test results yield Initial Reactive

   Molecular Confirmation test results
   - POSITIVE
     - Presumptive Positive
   - NEGATIVE

BAX

OR

Reveal

SDI

RapidChek

IMS

Marshfield Lab/

BioControl

Initial Reactive

AND

With

IMS

AND

Without

IMS

Presumptive Positive

Presumptive Positive
American Proficiency Institute (API)  
2004 to 2013

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Correct</th>
<th>Incorrect</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Rank</th>
<th>Overall Score (sensitivity plus specificity)</th>
<th>Overall Rank</th>
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<tbody>
<tr>
<td><strong>E. coli O157</strong></td>
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<td></td>
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<tr>
<td>Known +</td>
<td>802</td>
<td>800</td>
<td>2</td>
<td>99.8%</td>
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<td>#1/10</td>
<td>199.5</td>
<td>#1</td>
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<td>Known +</td>
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<td>429</td>
<td>3</td>
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<td>99.3%</td>
<td>#2/9</td>
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<tr>
<td><strong>L. spp.</strong></td>
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<tr>
<td>Known +</td>
<td>745</td>
<td>741</td>
<td>4</td>
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<td>#1/12</td>
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<td>#1</td>
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<tr>
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<td>204</td>
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<td>100.0%</td>
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<tr>
<td>Known +</td>
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<tr>
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<td>490</td>
<td>488</td>
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<td></td>
<td>99.6%</td>
<td>#1/12</td>
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</tbody>
</table>

**NOTE:** Salmonella: #1 specificity, USDA/FSIS, 33 for 33 - 100% (sensitivity #5/9; 96.3%)
Conclusions

- EHECs pose a serious threat to public health
- EHECs are likely to be present in Produce, Agriculture and other food products
- *E. coli* O157 is not the most predominant EHEC
- All EHECs should be considered, not just O157 or “Big Six”
- Make sure you are using the correct test method to detect EHECs
QUESTIONS?
CLOSING STATEMENTS