FSIS STEC Guidance:

Testing solutions to meet evolving regulations and consumer demand

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Millipore®

Preparation, Separation, Filtration & Monitoring Products

The life science business of
Merck KGaA, Darmstadt, Germany
operates as MilliporeSigma
in the U.S. and Canada.

Disclaimer

- The information in this seminar is given for the purposes of education and discussion.
- It is not intended to be, and it should not be used as a substitute for regulations or regulatory guidance.
- Decisions and actions should be based on the relevant regulations, guidance document and USDA and FDA chapters.
- Statements and opinions expressed are of the presenter and are not necessarily the views of MilliporeSigma.

01 Regulatory Landscape and Background

Justyce Jedlicka, Food & Beverage Regulatory Liaison and Field Marketing

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Background

November 2022
FSIS announcement of STEC testing and open comment period

November 2022
FSIS announcement of cessation of N60 excision sampling

January 2023
FSIS announced expansion to additional raw beef products

February 2023
FSIS implementation of new STEC testing

STEC - *Shiga Toxin Producing E. coli*

STEC Facts 2015 - 2019

12,866

Infections

- 22% hospitalized
- 15% travel-associated
- 5% outbreak-associated
- <1% died

July

Highest Month of incidents

- July trends 13.3% cases
- >10% infection rate occurs June - August

O157

Largest identified serotype

- Top 5 serotypes include
- CIDT+(not serogrouped) – 41.43%
- O157 – 17.44%
- O26 – 8.34%
- O103 – 8.29%
- Non-O157, not serogrouped – 6.96%

Minnesota

State with largest rate of infection

- Top 8 States
- Minnesota – 9.0
- California – 7.68
- Oregon – 6.78
- Colorado – 6.7
- Tennessee – 4.66
- New Mexico – 4.62
- Connecticut – 4.11
- Georgia – 3.66

https://wwwn.cdc.gov/foodnetfast/

FSIS Directive 10010.10 Revision 5

USDA **FSIS Directive 10,010.1 Dashboard** | *Sampling Verification Activities for Shiga Toxin-Producing Escherichia Coli (STEC) in Raw Beef Products*

TASK CODE

MT05 - Raw Ground Beef or Veal Sampling - Retail

Search posted Q/As with "askFSIS" in the title

Submit question to askFSIS

PREPARE

SCHEDULE THE SAMPLING TASK:

- Select correct assignment — Directive 13,000.2
- Filter for "Lab Sampling"
- Consider time needed for establishment to hold lot
- Find sample task on list, and select "Add"
- Schedule collection date

RECORDS TO COMPLETE:

- ** Notify plant management before collecting sample (i.e., hold sampled lot)*
- Lab Form Questionnaire as part of PHIS Task
  - Data specific to sample collection
- Supplier information (in case sample is positive) — Directive 10,010.3
  - Gather source material information when collecting a sample.

PERFORM

MT05 - Raw Ground Beef or Veal Sampling - Retail

Each MTxx Sampling Task is based on the "source material" and "finished product"

N60 Sampling Video | Cloth sampling video

SOURCE MATERIAL	PROJECT SUMMARY	SAMPLE METHOD
	Retail Firm (Compliance)	
	Ground beef from any source, sampled in the final package.	**2-lb Total**

RESPOND

COMPLETE THE SAMPLING TASK:

- Complete Lab Form Questionnaire
- Verify address in PHIS and on shipping label match
- Complete PHIS Task
- File supplier information for sample
- Verify product is held pending adulterant test result
- Wait for sample results (typically 2-3 days)

ACTIONS IN RESPONSE TO STEC PRESUMPTIVE POSITIVE:

- Review supplier and lotting information
- Verify all affected product is on hold until result is "confirmed"
- Await confirmation
- If confirmed positive, verify disposition
- If confirmed positive, verify HACCP requirements — Directive 10,010.2

USDA vs EU STEC Testing

US

- O26
- O45
- O103
- O111
- O121
- O145
- O157

- FSIS Directive 10010.1 - Sampling Verification Activities for Shiga Toxin-Producing Escherichia Coli (STEC) in Raw Beef Products - Revision 5
- BAM Chapter 4A: Diarrheagenic Escherichia coli

- Upcoming:
  - FDA Leafy Greens Action Plan
  - USDA 2025 Roadmap

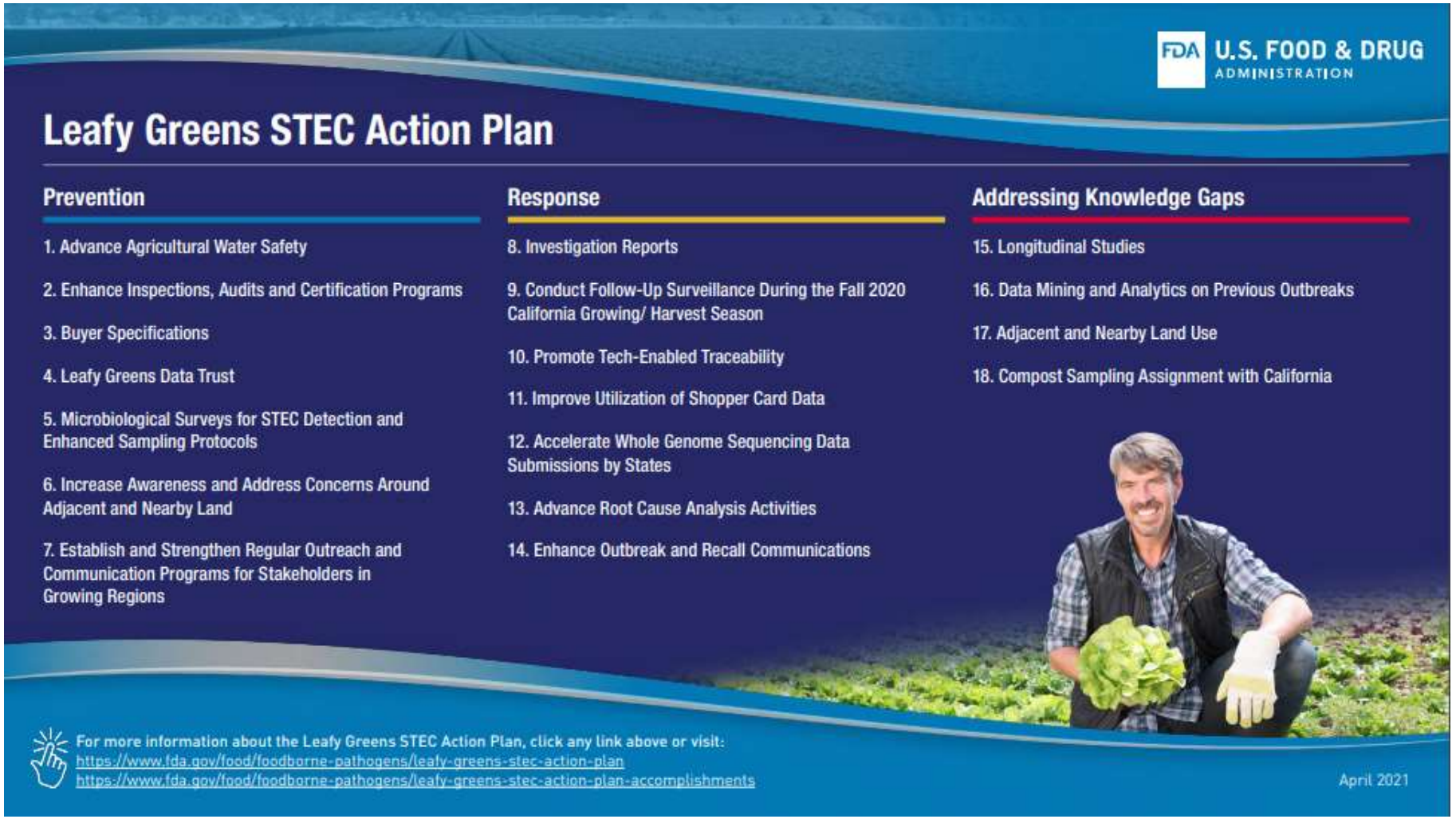
EU

- O26
- O103
- O111
- O145
- O157

- ISO/TS 13136 - Microbiology of food and animal feed — Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens — Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups

- Upcoming:
  - New Method
    - Detection of STEC
    - Serogroup / Virulence factor

FDA Leafy Greens Action Plan



https://www.fda.gov/food/foodborne-pathogens/leafy-greens-stec-action-plan

USDA Science Blueprint – 2025 Roadmap

Program Themes

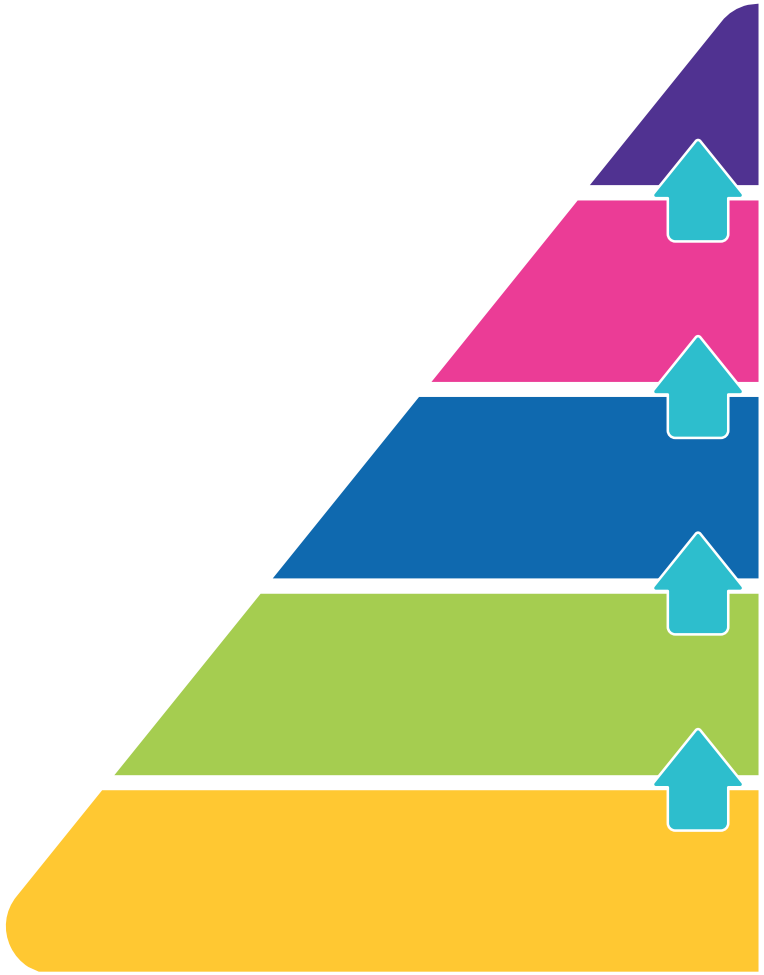
1. Sustainable Agricultural Intensification
2. Agricultural Climate Adaption
3. Food & Nutrition Translation
4. Value-Added Innovations
5. Agricultural Science Policy Leadership

Key Programs / Technology Development

1. Poultry: Salmonella & Campylobacter
2. Beef: STEC & Salmonella
3. Deli Meat: Listeria
4. Pork: Salmonella & Yersinia
5. Hemp
6. Expediting testing confirmation methods: WGS & MALDI

Organization

Hierarchy with five segments



Measure SP-1.1.2.1 Reduction in the proportion of poultry samples with Salmonella serotypes commonly associated with human illness

FSIS is analyzing comments for final rule stating intentions to expand non-O157 Shiga Toxin-producing Escherichia coli (STEC) testing to all beef products that are currently sampled for O157:H7

Agency continues to prioritize ready-to-eat (RTE) sampling for Salmonella and Listeria monocytogenes (Lm)

FSIS to evaluate whole genome sequencing complementary technology as part of initiative to Adopt Innovative Approaches and Technologies

FSIS will conduct further in-depth assessments of the sampling and testing protocols identified through the Strategic Assessment of Sampling Resources

02 Effective pathogen testing solutions: Assurance® GDS STEC

Michael Eastwood, Technology Specialist

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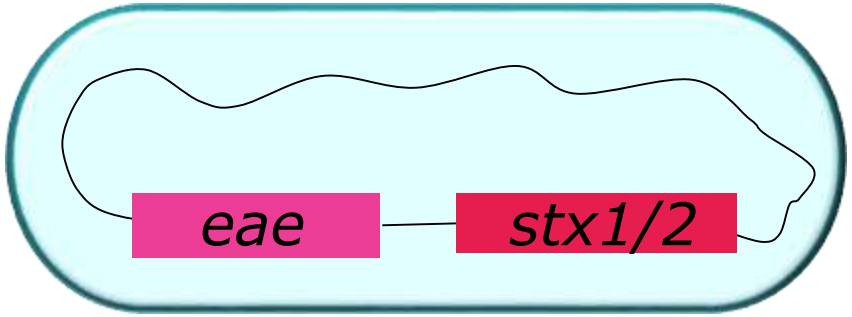
Accurately Screening For Top 7 STEC is More Difficult than *E. coli* O157

E. coli O157	PCR designed for O157-specific targets
	Most positives can be culturally confirmed
Top 7 STEC	Cannot achieve specificity with PCR design: cross-reactors inevitable
	Minority of positives are culturally confirmed*

* Bosilevac & Koohmarie 2012, App. Env. Micro

USDA Criteria for Top 7 STEC

+

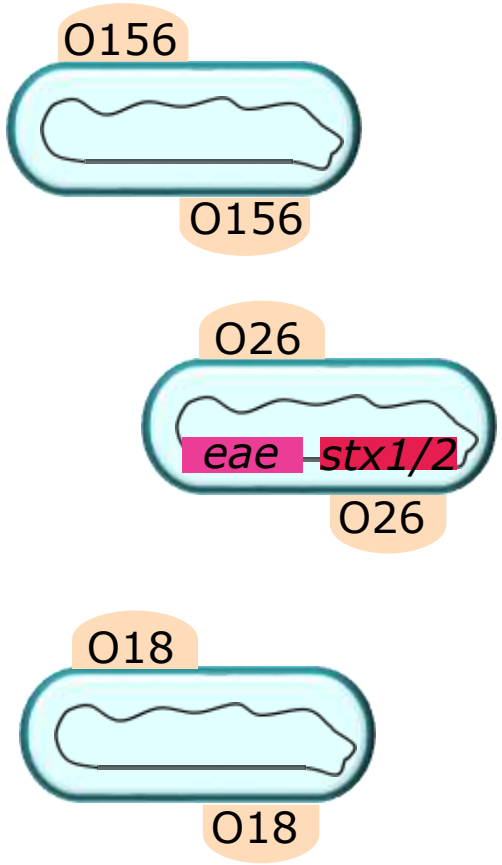


Immunological

Genetic

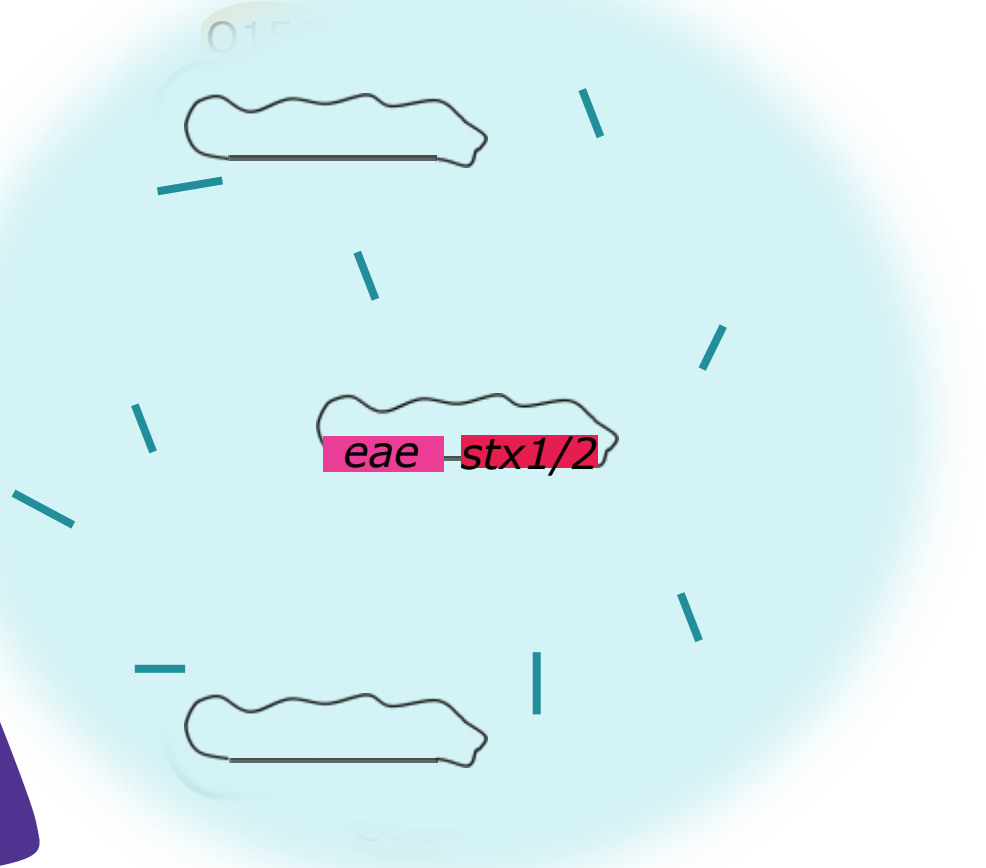
Conventional PCR Methods Do Not Detect if *eae* and *stx1/2* are in the Same Cell

Step 1. Enrich potential pathogens



Conventional PCR Methods Do Not Detect if *eae* and *stx1/2* are in the Same Cell

Step 1. Lyse cells to release pathogen DNA



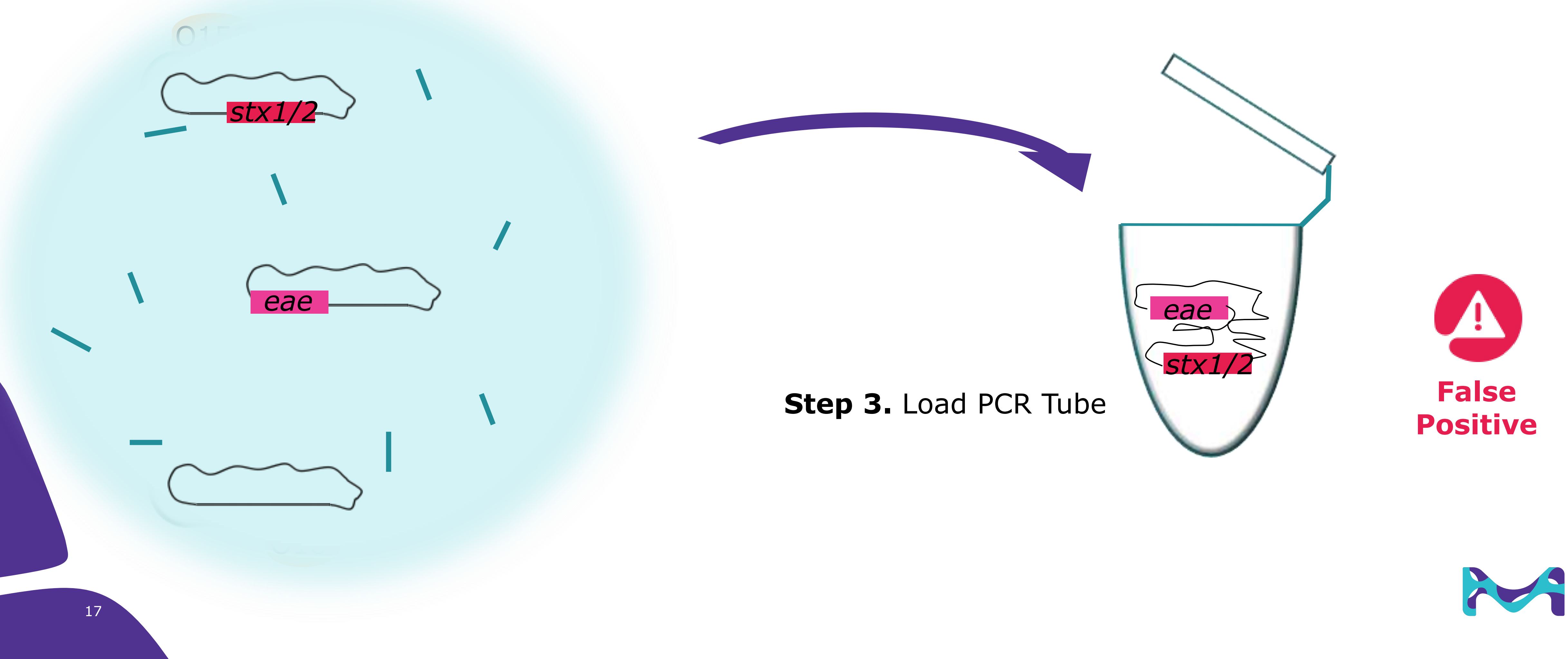
Step 2. Transfer lysate into PCR tube

Step 3. Load PCR Tube

Positive

Conventional PCR Methods Do Not Detect if *eae* and *stx1/2* are in the Same Cell

Step 1. Enrich potential pathogens
Step 2. Lyse cells into single pool



Assurance® GDS
What Is GDS?

Innovative PCR-based molecular pathogen screening method for food-borne pathogens

Developed to meet the daily rigors & requirements specific to food testing labs – adjustable throughput for small or large testing volumes

Designed to provide the most accurate and reliable results – all kits are AOAC validated across a wide range of matrices

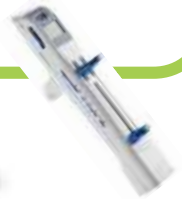
Assurance® GDS
Workflow Fundamentals

1

Enrichment

2

Reagents dispensing



PickPen® IMS

Transfer to Amp tubes

Manual procedure

Automated procedure

PickPen® PipetMax® (PPMX)

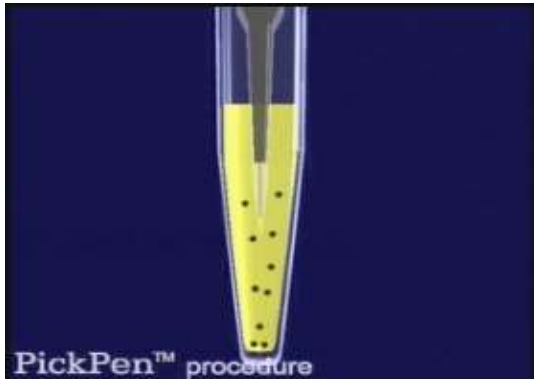
3

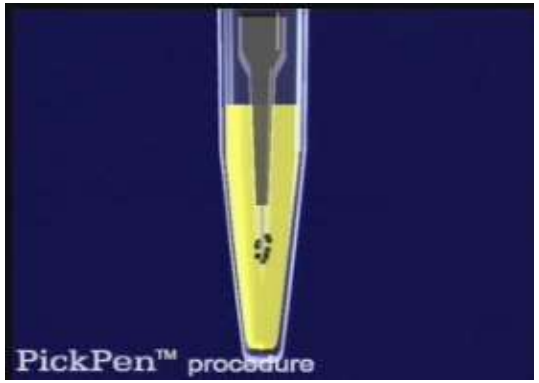
PCR analysis

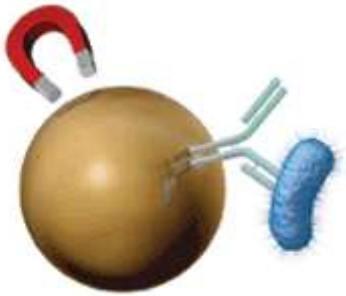
Assurance® GDS

How are we different?

PickPen® IMS





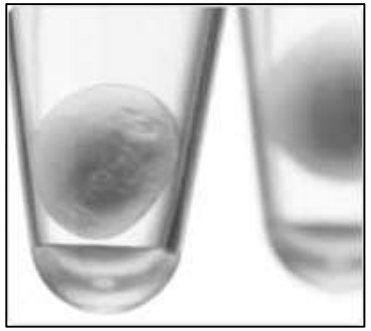


Proprietary “intrasolution” **Immuno Magnetic Separation (IMS)** using the PickPen®

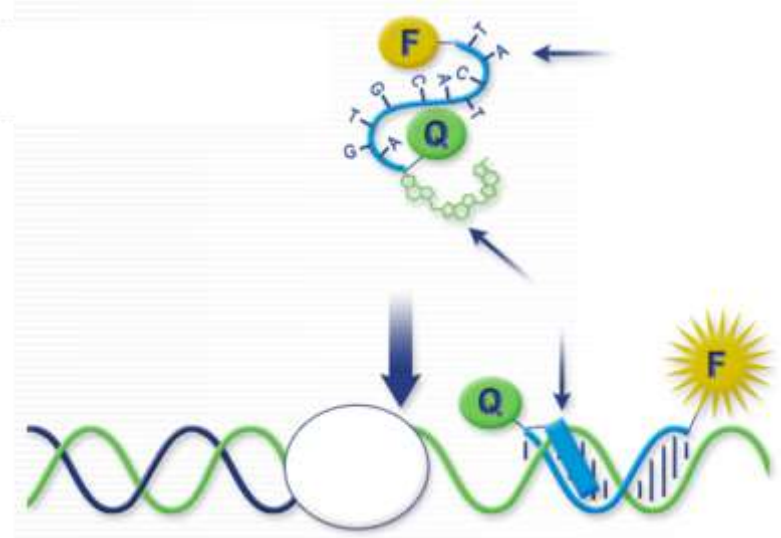
Assurance® GDS

DNA Target Amplification and Detection

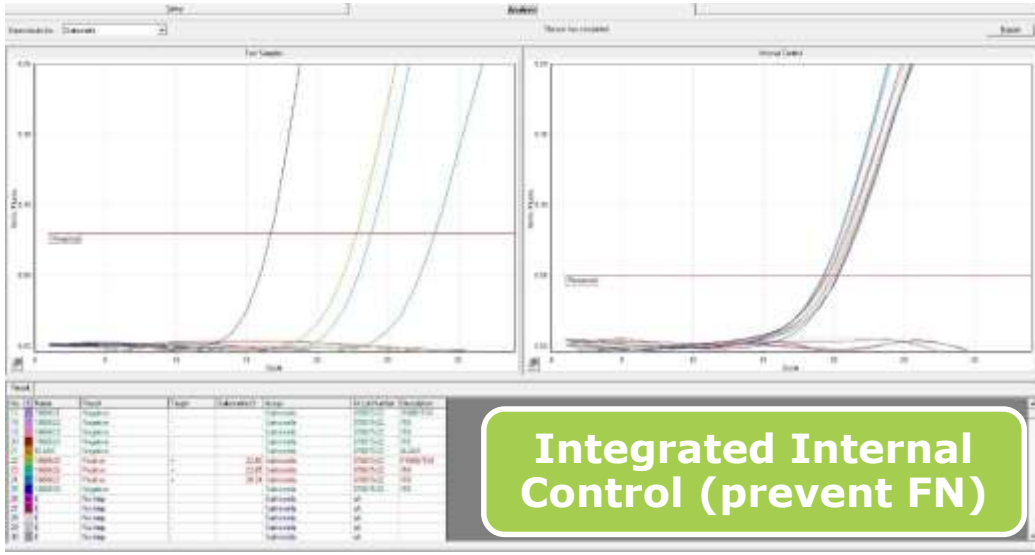
DNA Amplification with Lyophilized PCR Mix



Specific Detection via fluorescent probe



Automatic Analysis Software



Results

- Positive
- Negative
- No Amp

Assurance® GDS STEC Suite

Screening and Serogroup Identification Tools

Step 1: Top 7 STEC Screen

- PickPen® IMS selection of Top 7 STEC
- Differentiation of Big 6 STEC and O157

Step 2: Molecular Serogroup Identification

- Specificity through ***both*** serogroup and *eae*-subtype PCR targets

Assurance® GDS STEC Suite

Screening and Serogroup Identification Tools

Enrich mEHEC® Medium 10 Hours

Assurance® GDS STEC Suite

MPX Top 7 STEC Kit Screens for Virulence Genes in Top 7 STEC

Enrich 375g beef in mEHEC® for 10h

IMS of Top 7 STEC

Specific Detection of Virulence Genes

Immunological

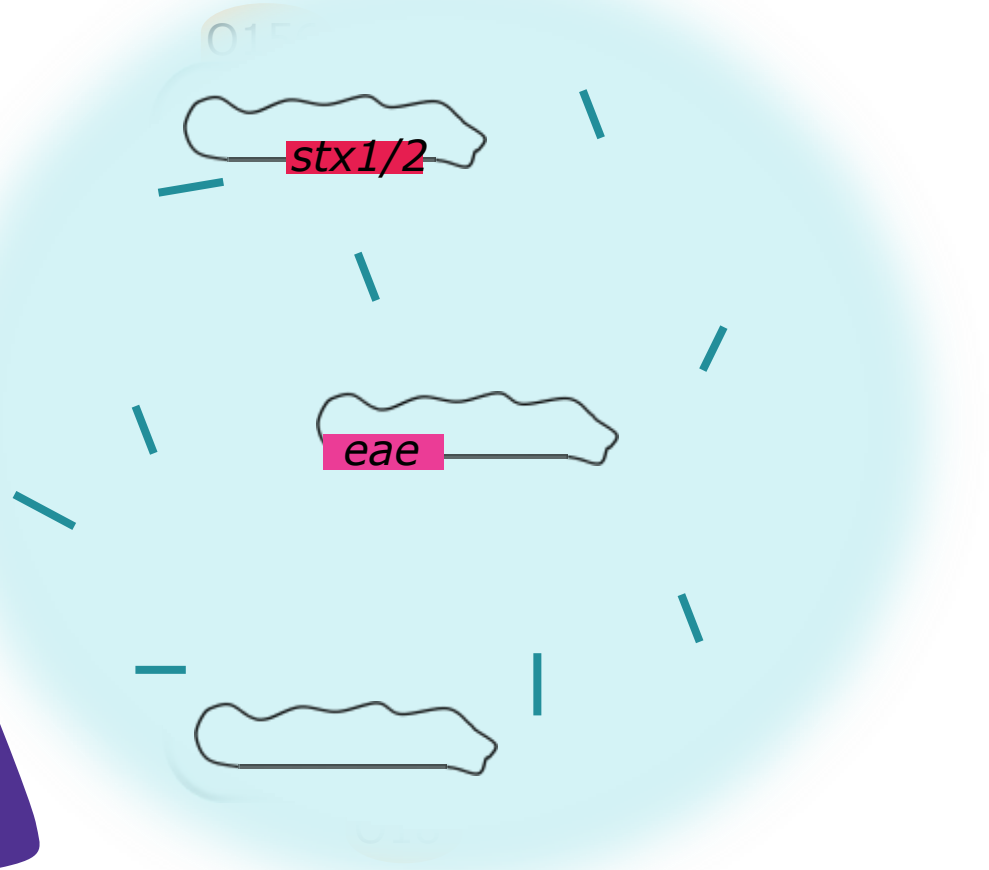
Genetic

- *E. coli* O157
- *eae*
- *stx1*
- *stx2*

Assurance® GDS STEC Suite

Conventional PCR Methods Do Not Detect if *eae* and *stx1/2* are in the Same Cell

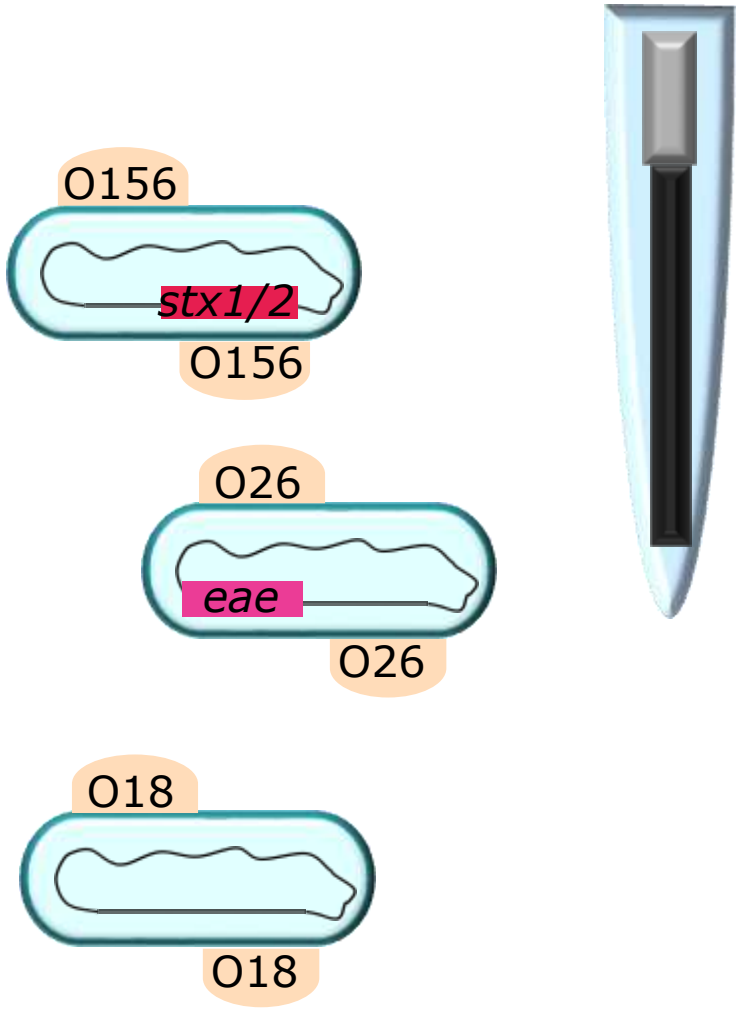
Step 1. Lyse cells to release genomes



Step 2. Load PCR Tube

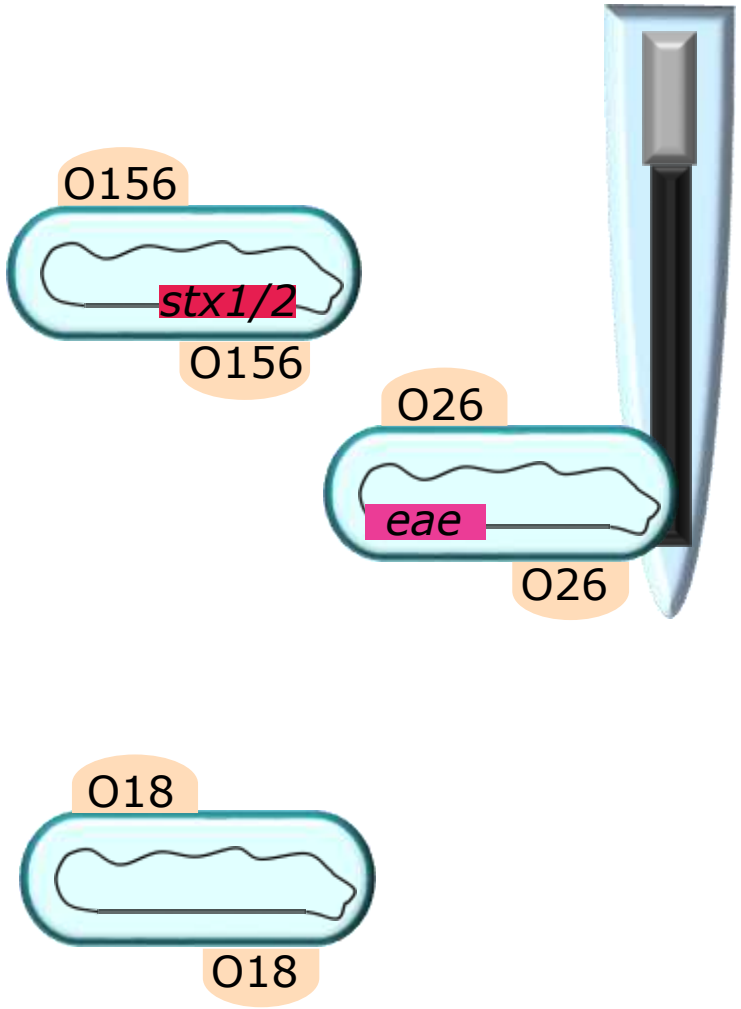
False Positive

Assurance® GDS STEC Suite
PickPen® IMS Enriches for Top 7 STEC



Step 1. Capture pathogens with PickPen®

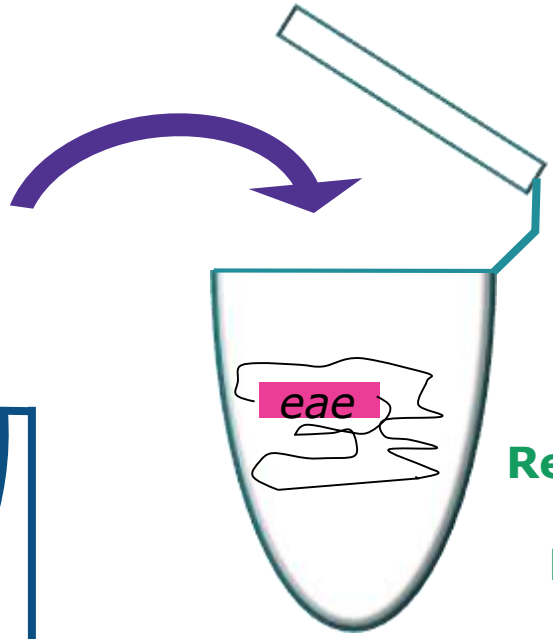
Assurance® GDS STEC Suite
PickPen® IMS Enriches for Top 7 STEC



Step 1. Capture pathogens with PickPen® beads

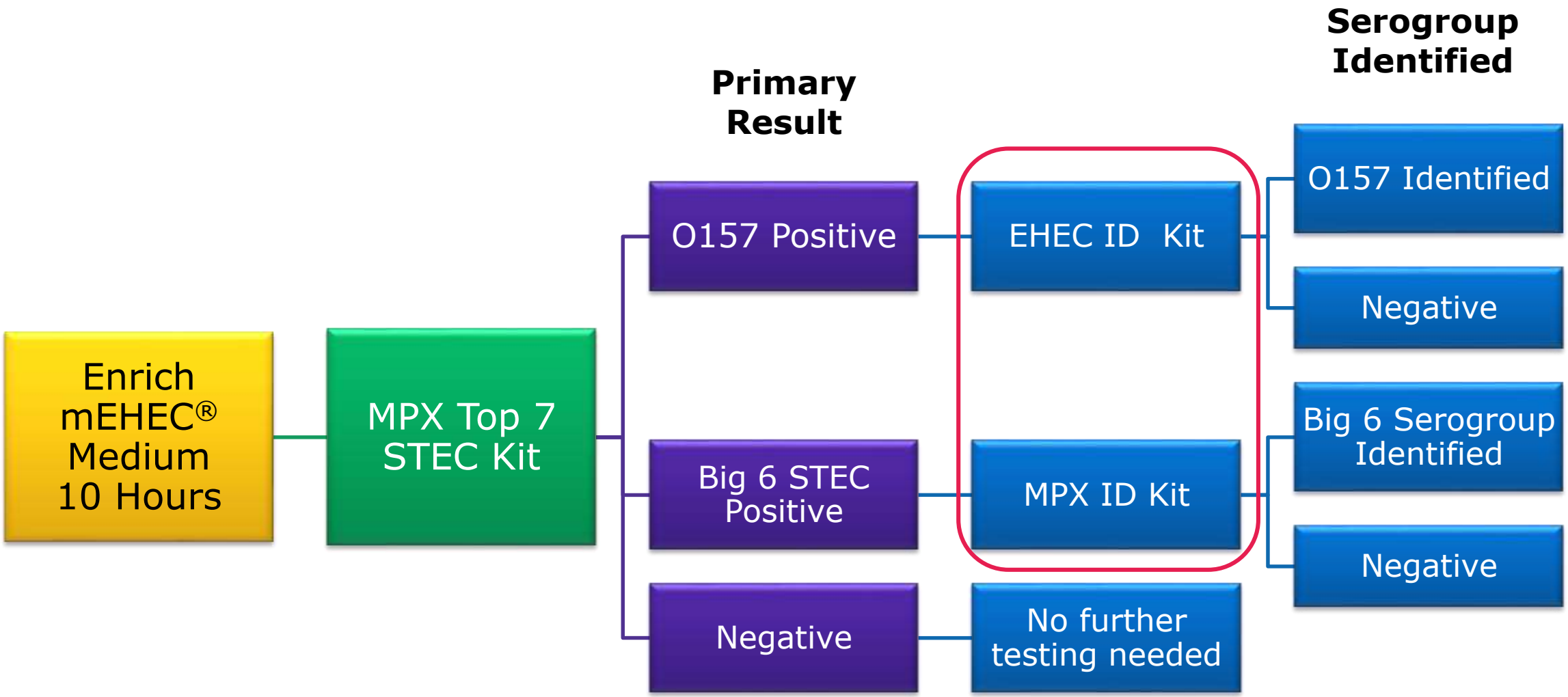
Step 2. Separate target pathogens

GDS Resuspension Well



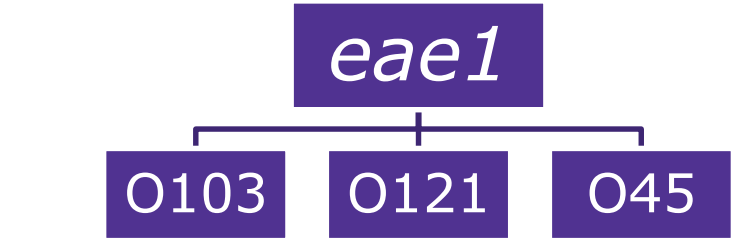
Reduction in False Positives

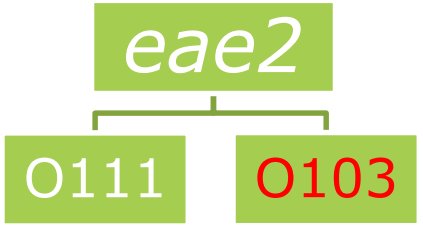
Assurance® GDS STEC Suite
Screening and Serogroup Identification Tools

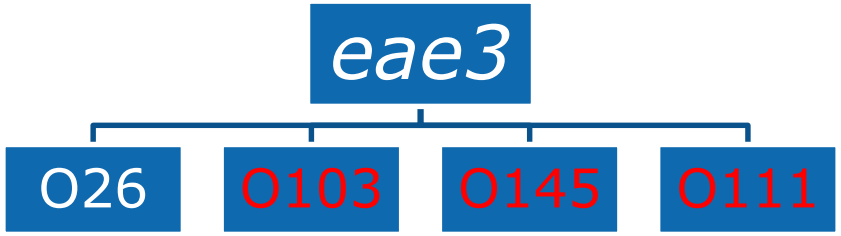


Assurance® GDS STEC Suite
MPX ID STEC Identification Kit

- Not just an O group assay
- 6 Non-O157 O groups are associated with specific *eae* subtypes
- Includes standard and atypical pairings







Assurance® GDS STEC Suite
MPX ID STEC Identification Kit

Big 6 Positive Sample

IMS of Big 6 STEC

- *eae*1 or ϵ
- *eae*2 or θ
- *eae*3 or β
- O103
- O121
- O45
- O26
- O111
- O145

STEC ID

Name	Top 6 STEC	O26	O45	O103	O111	O121	O145
O103, epsilon	Positive			+			
O121, epsilon	Positive					+	
O45, epsilon	Positive		+				
O103, beta	Positive			+			
O145, beta	Positive				+		+
O111, theta	Positive				+		
O26, beta	Positive	+					
NC	Negative						

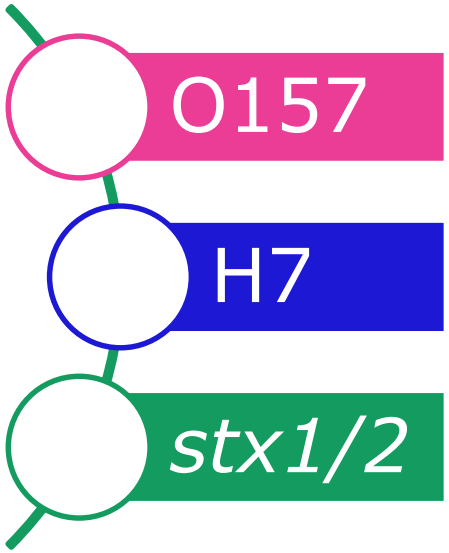
ID of O group simplifies isolation

Assurance® GDS STEC Suite
EHEC ID *E. coli* O157:H7 Identification Kit

O157 Positive Sample

IMS of *E. coli* O157

Detection of O157 Targets



Assurance® GDS STEC Suite
Summary