USDA Policy for Controlling VTEC: Regulatory and Methodological Aspects

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Discussion

- STEC = VTEC
- Serogroups, modes and vehicles of transmission
- STEC virulence
- Research projects
- New regulations
- Detection of STEC and related issues

Principal agencies in the U.S. Federal Government that deal with food safety

- U.S. Department of Agriculture
  - **Food Safety and Inspection Service (FSIS):** ensure the safety of meat, poultry, and egg products
  - **Agricultural Research Service:** Principal in-house research agency of the USDA (food safety, global climate change, bioenergy, food animal production, etc.)

- Department of Health and Human Services
  - **Food and Drug Administration (FDA):** ensure the safety of domestic and imported foods marketed in interstate commerce
  - **Centers for Disease Control and Prevention (CDC):** food safety - surveillance and investigation of illnesses associated with food consumption

Estimates of annual STEC infections

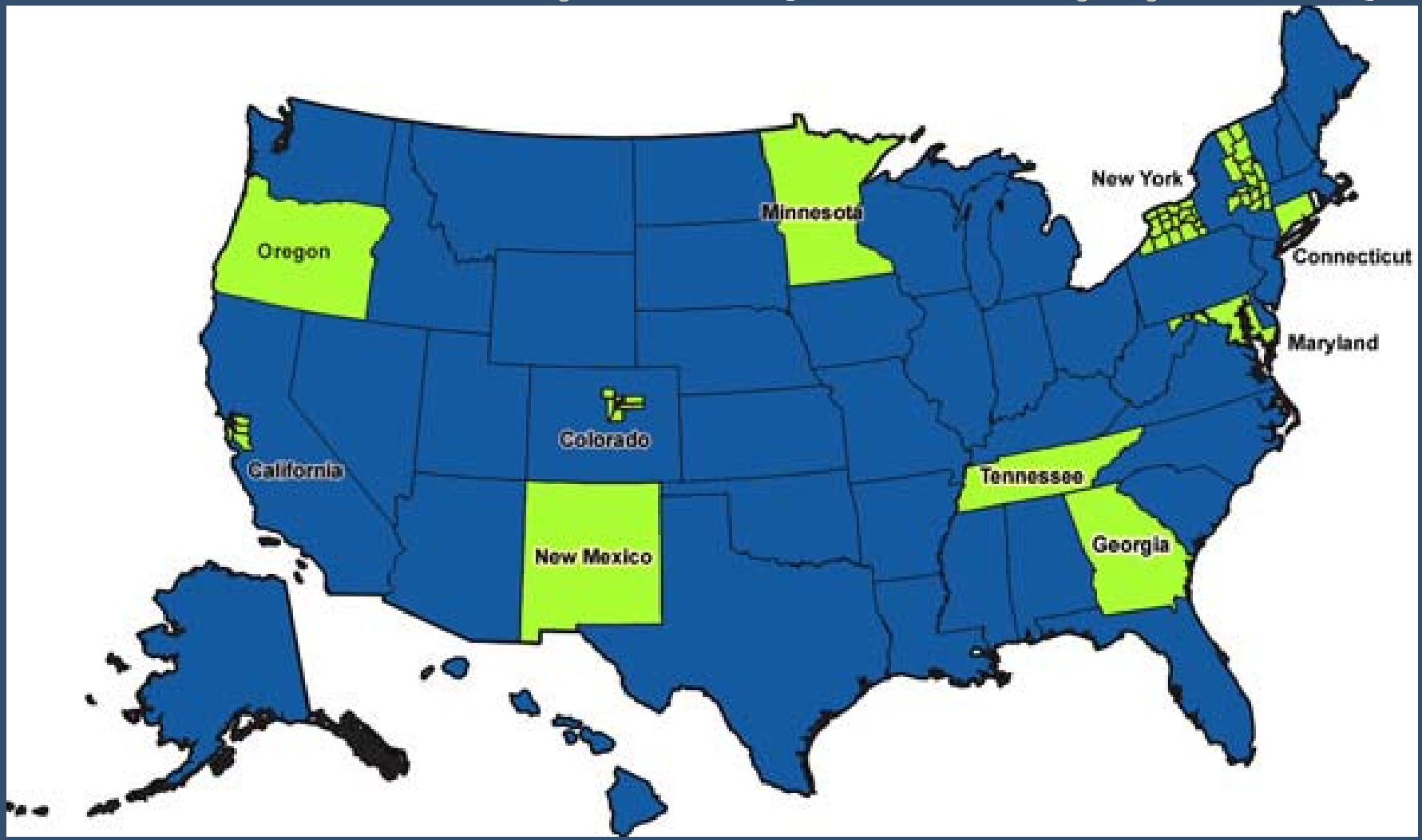
	STEC O157 (90% CrI)*	Non-O157 STEC (90% CrI)	Total
All infections	96,000 (27,000–228,000)	169,000 (17,000–428,000)	265,000
All hospitalizations	3,300 (800–7,000)	400 (0–1,400)	3,700
All deaths	30 (0–170)	0 (-)	30
Foodborne infections	63,000 (18,000–150,000)	113,000 (11,000–287,000)	176,000

Scallan et al. 2011. Emerg. Infect. Dis. 17:7-15 - all numbers rounded

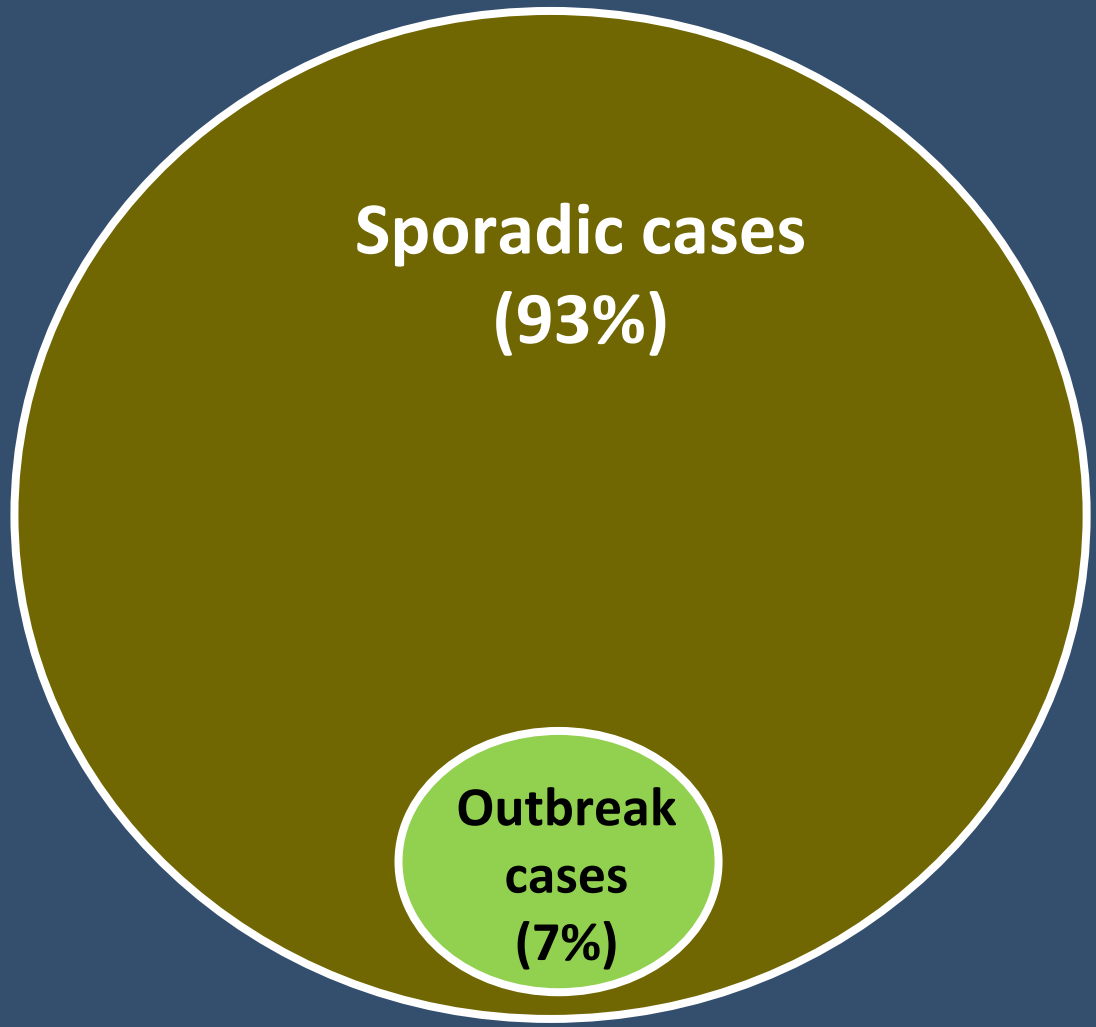
*90% credible limits

Food Net

10 sites , 46 million persons (15% of US population)

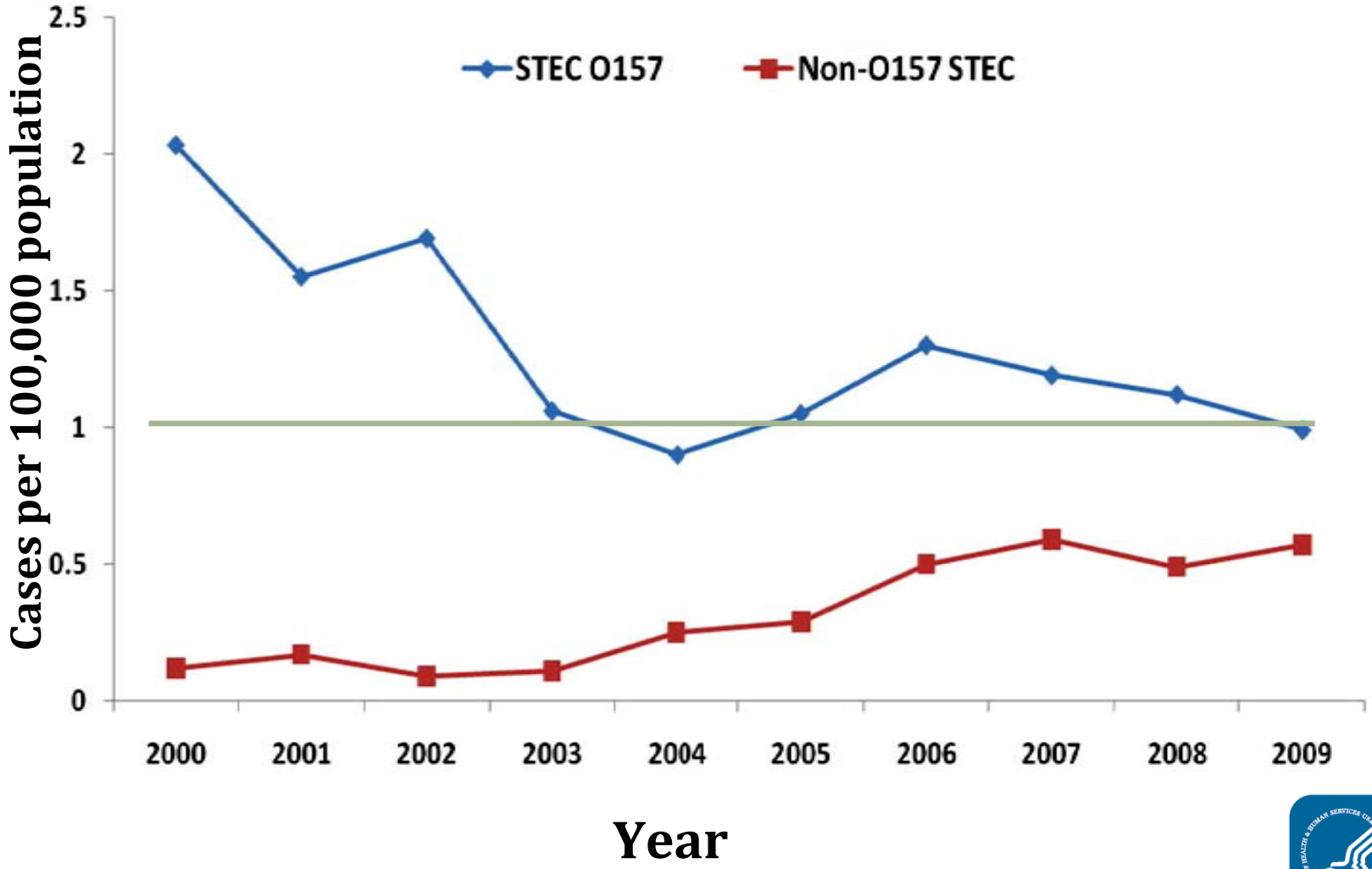


Most non-O157 STEC cases are not part of recognized outbreaks*

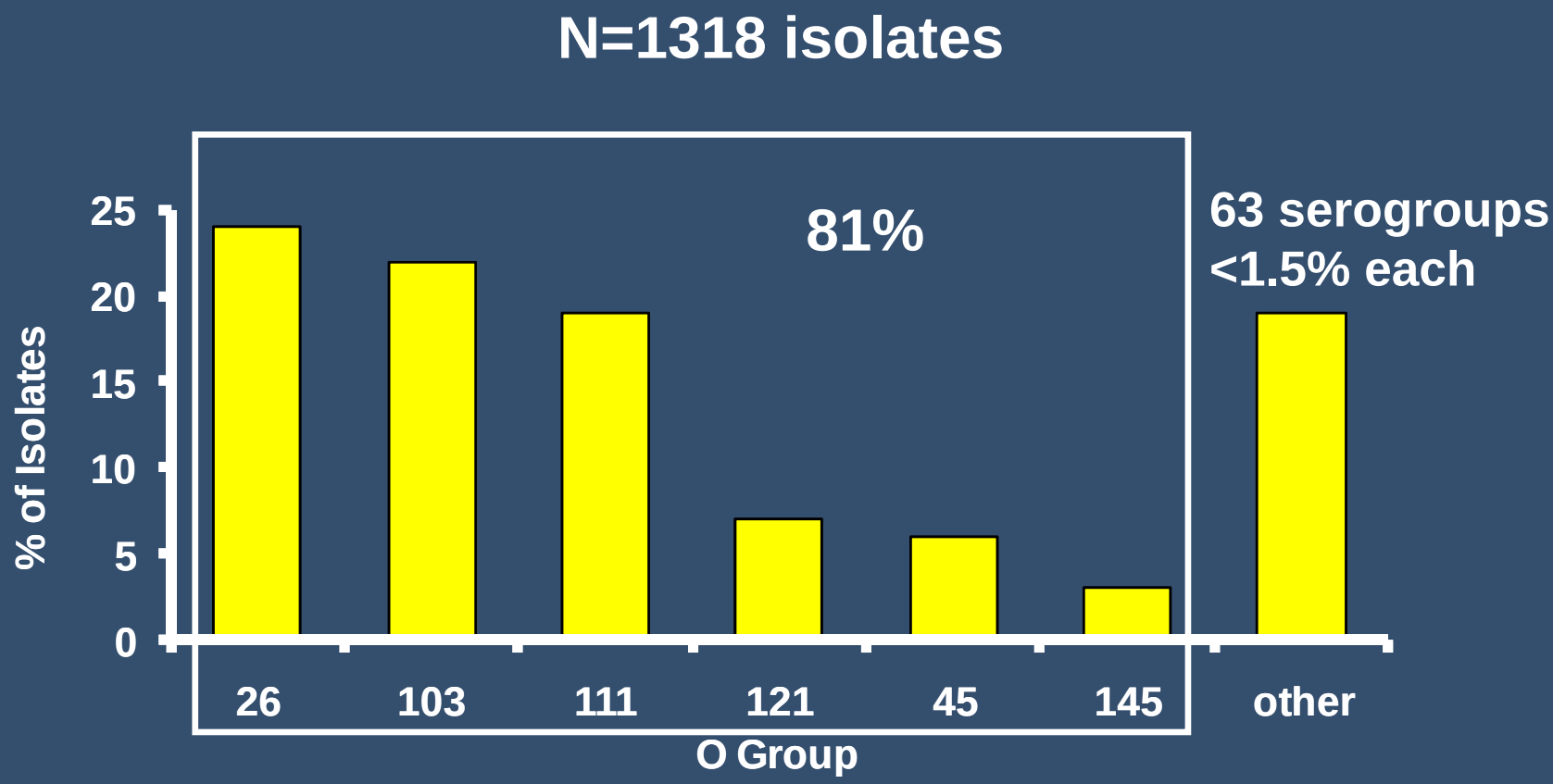


*FoodNet, 1301 total cases from 2004-2009

Incidence of reported STEC O157 and non-O157 STEC infections, by year, FoodNet, 1996-2009



Non-O157 STEC by serogroup FoodNet, 2000-2009



Preliminary data; an additional 85 isolates had missing O group information

Serogroups in non-O157 STEC outbreaks, United States

Serogroup	Single-etiology outbreaks no.	Multiple-etiology outbreaks* no.	Total outbreaks** no.
O111	15	6	21
O26	12	2	14
O121	5	2	7
O45	4	0	4
O145	2	1	3
O104	1	0	1
O103	1	0	1
O165	1	0	1
O84	0	1	1
O51	0	1	1
O141	0	1	1
Undefined	0	2	2

* Other pathogens in multiple etiology outbreaks included *Cryptosporidium, Campylobacter,* STEC O157, *Salmonella, Shigella,* rotavirus and norovirus

**Total is >52 because in some mixed-etiology outbreaks had more than 1 serogroup isolated

Modes of transmission

	Single-etiology (n=41)		Mixed-etiology (n=11)	
	Outbreaks	Illnesses	Outbreaks	Illnesses
Mode	**%**	**%**	**%**	**%**
Person-to-person	39	17	27	2
Foodborne	**34**	**63**	**36**	**65**
Water	5	2	18	22
Animal contact	2	1	18	11
Mixed	7	16	-	-
Unknown	12	2	-	-
Total	**100**	**100**	**100**	**100**

Commodities in 14 single-etiology foodborne outbreaks, 1994–2010*

Commodity	Outbreak no.	Food items
Dairy	2	pasteurized & unpasteurized milk
Leafy vegetable	2	lettuce
Beef	2	ground beef
Pork	1	barbeque pork
Game meat	1	bear jerky
Fruits-nuts	1	berries
Unclassifiable	1	punch
Unknown	4	? (2 with ill food workers)

*First reported foodborne outbreak of non-O157 STEC infection in the United States was in 1994.

Animal reservoirs

- Cattle
- Sheep, goat, buffalo, guanaco, deer, elk
  Non-ruminants: cat, dog, pig, horse, rabbit, poultry
- "Transport" hosts; birds, rodents, flies, beetles
- Sources of STEC: food, water, contact with infected animals or their environment

- **Bosilevac and Koohmaraie. 2011. Appl. Environ. Microbiol.77:2103-2112**
  - N=4133 ground beef samples, 2-year period
  - 24% → *stx*$_{1/2}$ positive; isolation from 300 samples → 7.3% of N=4133 (incl. O26, O103, O121, O145)
  - 10 isolates were potential pSTEC (O26, O103, O145)

Non-O157 STEC outbreaks

http://bites.ksu.edu/sites/default/files/non0157outbreaks.pdf

- **August 2008 – STEC O111:NM, *stx*$_1$- *stx*$_2$**
- 341 cases
- 71 hospitalizations
- 26 HUS (8%)
- 1 death

- **April 2010 – STEC O145, *stx*$_2$- e*ae***
- ~34 cases in 5 states
- 40% hospitalized, 10% developed HUS

- **June 2010 – STEC O26, *stx*$_1$- *eae***
- Cases in 2 states, Maine and New York (3 illnesses)

- **October 2010 – STEC O26**
- Child care center , 9 diarrhea, 1 bloody diarrhea

STEC virulence genes

Virulence Factors in STEC	Gene Location
Shiga toxin (*stx)*	Phage
Intimin (*eae)*	PAI (LEE)
Enterohemolysin (*ehx/hly*)	Plasmid
Non-LEE effectors (*nle*)	PAIs, O-islands
Saa adhesin (STEC agglutinating adhesin)	Plasmid
Subtilase cytotoxin (*subAB*)	Plasmid
ToxB adhesin	Plasmid

- Other fimbrial and non-fimbrial adhesins, proteases (EspP, KatP), toxins, e.g., encoded by *cnf, astA, cdt*

- Acid tolerance

STEC virulence

- **Karmali et al. 2003.** Association of **genomic O-island 122** of *Escherichia coli* EDL 933 with verocytotoxin-producing *Escherichia coli* seropathotypes that are linked to epidemic and/or severe disease. J. Clin. Microbiol. 41:4930
  - Seropathotypes A-E *A:* O157:H7/NM (OI-122- 100%)
    B: O26, O103, O111, O145 (OI-122 – 60%)

- **Coombes et al. 2008.** Molecular analysis as an aid to assess the public health risk of non-O157 Shiga toxin-producing *Escherichia coli* strains. Appl. Environ. Microbiol. 74:2153
  - Molecular risk assessment
    - 16 *nle* genes encoded on O-islands 36, 57, 71, and 122
    - *nleB* present in all seropathotype A and B strains → HUS
    - Additive *nle* gene content → more severe disease
    - OI-122, OI-57, and OI-71 more often associated with HUS strains

STEC virulence genes

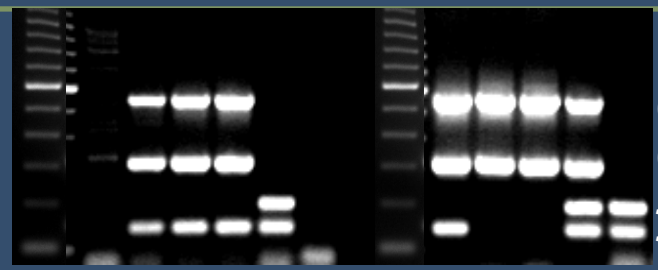
- Shiga toxins
  - Stx (*Shigella*), Stx1a (Stx1), Stx1c, Stx1d
  - Stx2a (Stx2), Stx2b-(Stx2d), Stx2c, Stx2d-(Stx2d-activatable), Stx2e, Stx2f, Stx2g)
- Eae (intimin outer membrane protein – AE lesion)
  - A number of variants/subtypes (alpha, beta, gamma, etc.)
- *eae* variants found in specific STEC serotypes
  - O157:H7 –*eae* gamma-1
  - O26:H11 –*eae* beta-1
  - O45:H2/H¯ –*eae* epsilon-1, beta-1
  - O103:H2 –*eae* epsilon
  - O111:H8 –*eae* theta
  - O121:H19 –*eae* epsilon
  - O145:H28 –*eae* gamma-1

Non-O157 STEC genes associated with illness

- *stx*$_2$ and *eae* → increased risk of HUS
- stx$_{2a/2c/2d}$ → associated with more severe disease (HC, HUS)
- All Shiga toxin subtypes associated with diarrhea; *stx*$_1$, *stx*$_{1c}$, *stx*$_{2e}$ also –associated with HC and HUS

Research on *E. coli*, non-O157 STEC

- Molecular serotyping
  - *E. coli* O-antigen gene clusters
  - Serogroup-specific sequences



STEC O145

- O157:H7 and non-O157 STEC detection methods

- STEC in swine
  - Prevalence, serotypes, virulence genes

- Gene expression of O157:H7 in ground beef
  - Fratamico et al. 2011. Differential gene expression of *E. coli* O157:H7 in ground beef extract compared to tryptic soy broth. J. Food Sci. 76:M79-87.

- Acid tolerance in STEC

O157:H7 and non-O157 STEC: adulterants

- *E. coli* O157:H7 causes life-threatening illness when food is prepared ordinarily
  - FSIS declared this form of STEC an adulterant in certain raw beef products in 1994 and further clarified the adulteration circumstances in 1999 and 2002
    - Initially associated with illnesses from consumption of ground beef; later applied to beef trimmings (intact source materials used for non-intact purposes such as ground beef) and to tenderized beef (non-intact beef)
- 2000: non-O157 STEC nationally-reportable
  - Cases reported to FoodNet increasing
  - Citizen's petition to FSIS – 2009 (Marler Clark law firm)
  - Senator Kirsten Gillibrand introduced new legislation to test for non-O157 serogroups in meat

STEC contamination of beef – focus on prevention

- **Illness Associated with Food**
  - Potential for illness if STEC is present at low number / serving
  - STEC may not be destroyed by ordinary cooking
- **A Primary Source is Beef**
  - Raw beef appears to be the primary source of STEC in foods regulated by FSIS
- The Administration, through the Food Safety Working Group, has focused on prevention as the fundamental principle for building a modernized food safety system
- Key component of this focus on prevention is the need for proactive strategies to reduce the number of food-borne outbreaks

Non-O157 STEC - adulterants

- Elizabeth Hagen: "We'll begin testing beef trim initially because that's where we get the biggest bang for our buck," Dr. Hagen told reporters. Beef trim is used to make ground beef.

"The impact of foodborne illness on a family can be devastating, Consumers deserve a modernized food safety system that focuses on prevention and protects them and their families from emerging threats. As non-O157 STEC (Shiga toxin-producing *E. coli*) bacteria have emerged and evolved, so too must our regulatory policies to protect the public health and ensure the safety of our food supply."

- Tom Vilsack emphasized that USDA was acting preemptively of waiting for a devastating outbreak.

"Too often, we are caught reacting to a problem instead of preventing it," said Vilsack. "This new policy will help stop problems before they start."

New non-O157 STEC policy

- As announced on September 20, 2011, FSIS plans to begin testing beef manufacturing trimmings on March 5, 2012, for six non-O157 STECs (O26, O103, O111, O121, O45, AND O145) – see:

http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/2010-0023.pdf

- FSIS plans to begin testing other raw beef for the six non-O157 STECs as FSIS laboratory capacity is established (a Federal Register Notice will announce implementation plans prior to March 2012)
- FSIS made a risk profile on the six non-O157 STECs available – see:

http://www.fsis.usda.gov/PDF/Non_O157_STEC_Risk_Profile.pdf

- FSIS also made a guidance document on test kit validation available – see:

http://www.fsis.usda.gov/PDF/Validation_Studies_Pathogen_Detection_Methods.pdf

Cost of program

- FSIS beef testing – non-O157 STEC

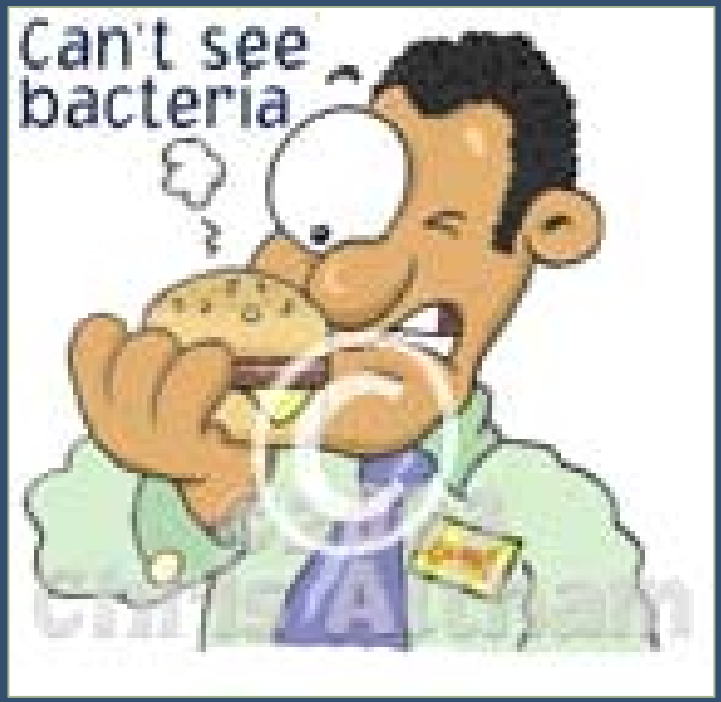
- Cost to FSIS: $454.9K to $730.8K
  - Laboratories sampling and testing: around $204K to 338.3K
  - for- cause FSA: about $180.5K to $322.1K

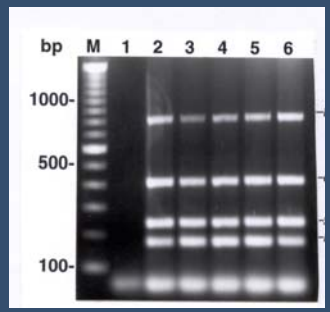
- Costs to the industry
  - establishments to start their own screening and testing : $4 to $5.3 million
  - diverting the products to cooking when the test result is positive: $3.9 to $5.2 million

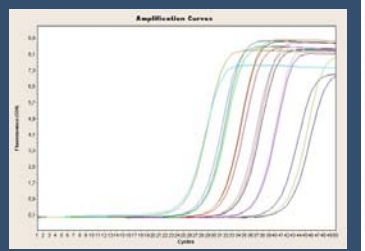
Benefits of program

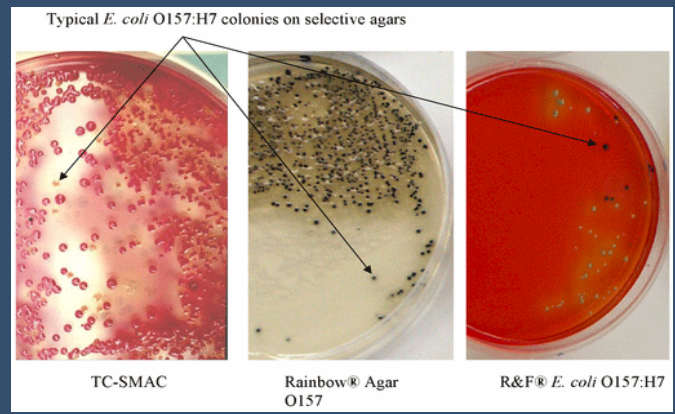
- Reduced illness and death
- Avoided recalls: each avoided recall is a saving of $3 to $5 million (based on FDA estimate of Class I recall costs)
- Caveat: high degree of uncertainty, especially number of illnesses reduced by testing
- FSIS has determined that potential public health benefits justify the costs

Detection of non-O157 STEC









Detection of non-O157 STEC

- Genetic variability in non-O157 STEC
  - Enrichment: selective agents
  - Screening assay: target genes
  - Isolation: selective and differential medium
  - Immunomagnetic separation and latex reagents: antibodies to specific serogroups

Detection of non-O157 STEC

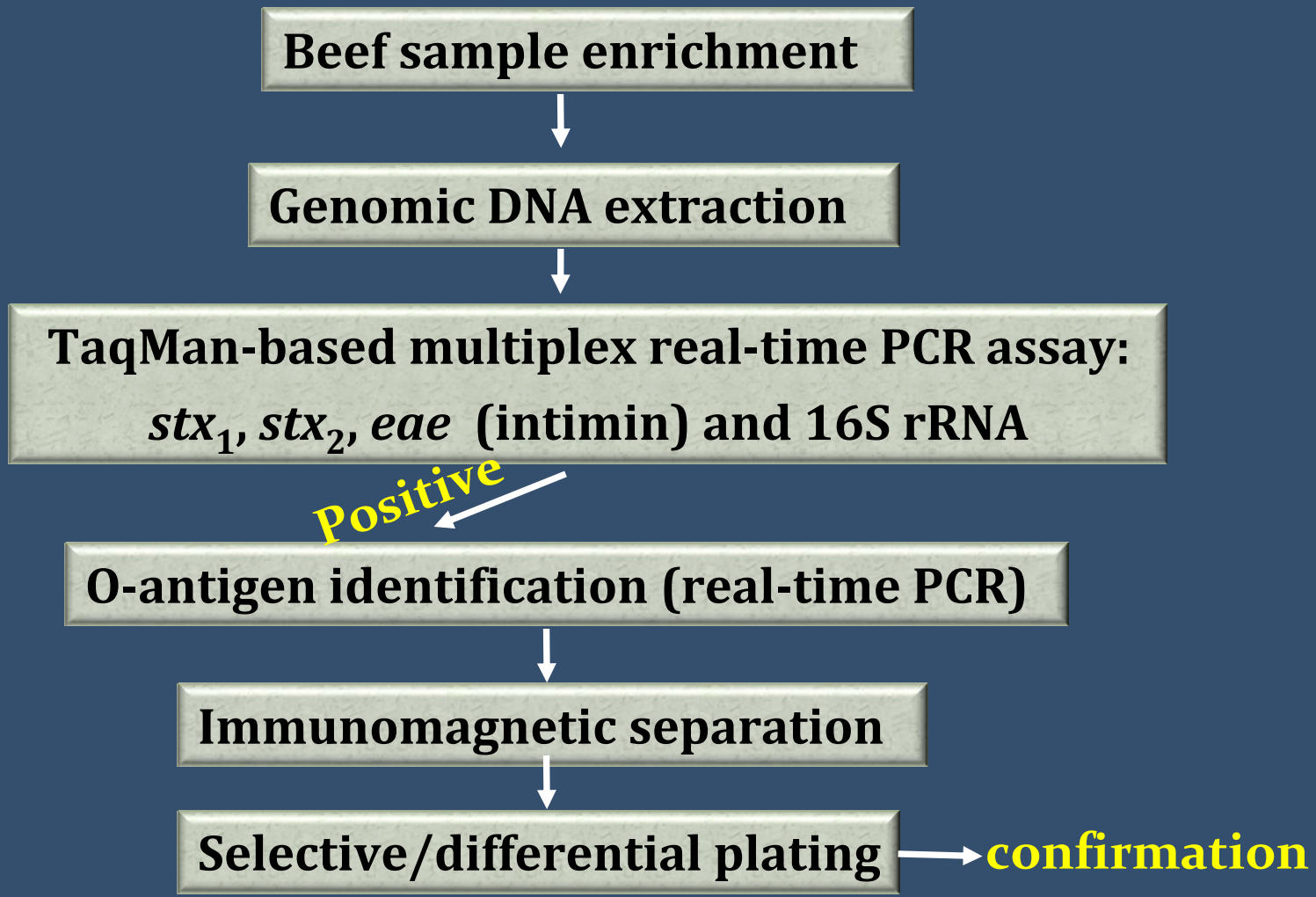
- **MLG 5B.00**

  Detection and Isolation of non-O157 Shiga-toxin Producing *Escherichia coli* Strains (STEC) from Meat Products (Effective Date: 10/01/10)

  http://www.fsis.usda.gov/PDF/Mlg_5B_00.pdf

- Fratamico et al. 2011. Detection by multiplex real-time polymerase chain reaction assays and isolation of Shiga toxin–producing *Escherichia coli* Serogroups O26, O45, O103, O111, O121, and O145 in ground beef. Foodborne Pathog. Dis. 8:601-607.

Detection of non-O157 STEC



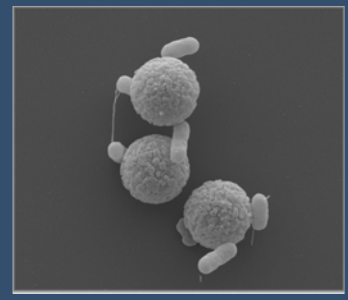
Detection of non-O157 STEC: MLG 5B.00

- Enrichment
  - O157 and non-O157 STEC
  - 325±32.5 g beef -- 975±19.5 ml mTSB + novobiocin and casamino acids
  - 42±1 for 22-24 h

- Multiplex PCR assays
  - *stx*$_{1/2}$ and *eae*, internal control (16S rRNA)
  - Serogroup-specific, *wzx* gene
    - O26 and O111 – internal control (IC)
    - O145 and O103 – IC
    - O45 and O121 - IC

Immunomagnetic separation and isolation

- Dynabeads® EPEC/VTEC O26
- Dynabeads® EPEC/VTEC O103
- Dynabeads® EPEC/VTEC O111
- Dynabeads® EPEC/VTEC O145



- **IMS for *E. coli* O121 and *E. coli* O45**
  - Polyclonal anti-*E. coli* O121 and anti-*E. coli* O45 typing serum → isolation of IgG → link to biotin → coat streptavidin-coupled Dynabeads

- Plating onto Rainbow Agar O157
  - 0.8 mg/L potassium tellurite, 10 mg/L novobiocin

Non O157 STEC in Meat Products

Day 1

Per 325g sample, add 975ml of mTSB+n [1], stomach for 2 min. Incubate @ 42 ± 1°C for **22-24 hours**

* This chart represents the best-case scenario but analyses may take longer due to normal analytical circumstances such as restreaking isolates for purity. If original 5 picks are negative an additional day may be required to process up to 10 additional picks. If the thermocycler capacity is exceeded additional days may be required to continue analysis.

Day 2

DNA Extraction

Multiplex RT PCR (*eae*, *stx*)

→ Either ***stx*** **(-)** or ***eae*** **(-)** Report as **NEGATIVE**

eae **(+) &** ***stx*** **(+)**

Multiplex RT PCR (*wzx* genes)

→ ***wzx*** **(-)** Report as **NEGATIVE**

wzx **(+)** → Report as **Potential (+)**

Prepare aliquot of mTSB+n for Immunomagnetic capture for 1 of 6 serogroups **(O26, O45, O103, O111, O121, O145)**

→ Streak prepared mTSB+n to Rainbow Agar and incubate @35°C, 20-24hrs

Day 3

Select typical well-isolated colonies from Rainbow Agar.

Negative or atypical colonies → Report as **NEGATIVE**

* Pick 15 typical colonies, streak 5 to SBA[4] and streak 10 to gridded SBA[4] or BHI[5] plate, and streak for purity then incubate @35°C for 18-24hrs.

DNA Extraction

1 Modified Tryptone Soya or Trypticase Soy Broth with novobiocin plus casaminoacids
2 Laboratory Electronic Application For Results Notification
3 Biological Information Transfer & E-mail System
4 Sheep Blood Agar
5 Brain Heart Infusion Agar
6 Outbreak Section of the Eastern Laboratory, USDA-FSIS

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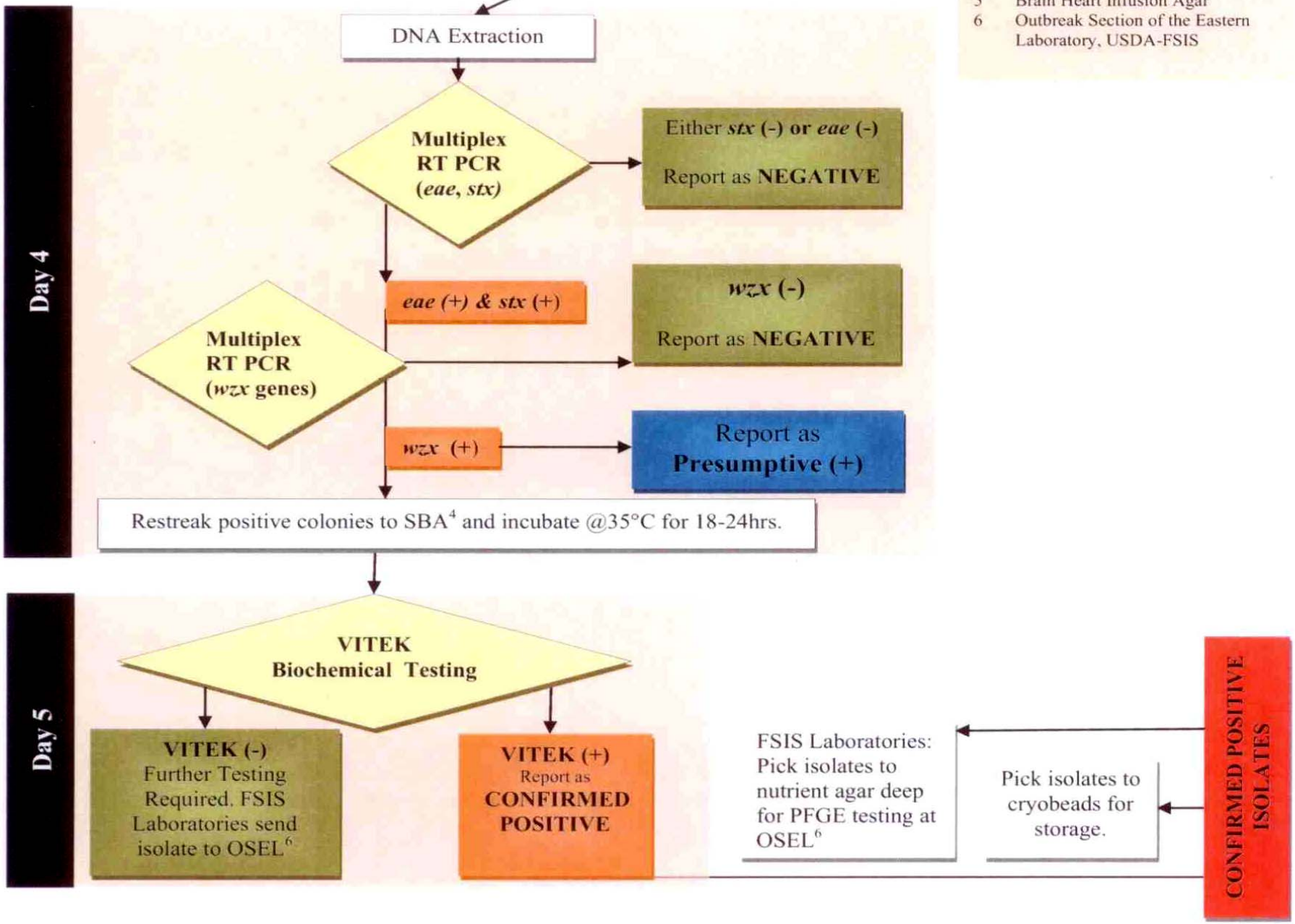
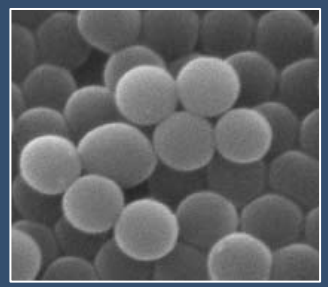


Latex reagents and control strains

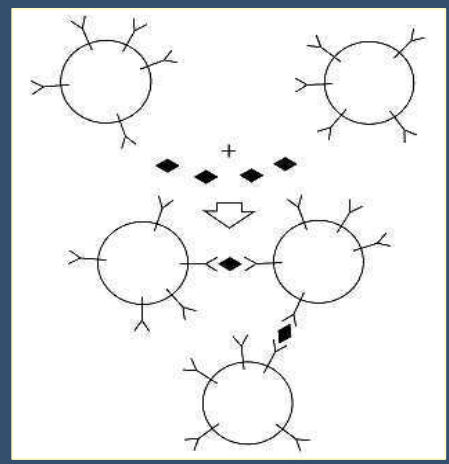
- Latex reagents



Latex beads

+

IgG reactive to O groups O26, O45, O103, O111, O121, and O145



Agglutination of presumptive positive non-O157 STEC colonies

- Non-O157 STEC method control strains

E. coli target gene

Unique genetic marker

Revised MLG - MLG 5B.01

http://www.fsis.usda.gov/Science/Microbiological_Lab_Guidebook/index.asp

- Enrichment
- Primers – probes
  - Sample will then be "potential positive" if it tests positive for the *stx* gene and the *eae* gene and is also positive for one or more of the target O-group genes
- IMS procedure
- Plating medium
- Use of latex reagents
  - Typical colonies are tested with latex agglutination reagents specific for the target serogroup
  - If at least one colony tests positive by latex agglutination, the sample is "presumptive positive"
  - A "presumptive positive" isolate that, upon further characterization by biochemical test, is *E. coli* , is a "confirmed positive" sample

Commercially-available non-O157 STEC assays

- **BioControl Systems**
  - Top six non-O157 STEC and O157:H7 (Assurance GDS)
  - IMS-based sample preparation targeting top six serogroups followed by PCR targeting *eae*, stx_1, stx_2
- **Pall Corporation**
  - AOAC validated method for detection of non-O157 STEC (O26, O103, O111, O145) in beef - GeneDisc
- **BioGX**
  - *Multiplex for CDC "Big Six" Non-O157 STEC Serotypes*
  - Lyophilized sample-ready reagents (O145, O111, O26; O45, O103, O121; stx_1, stx_2 and *eae*, *uidA* or *wzy*)
- Others available in near future

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Thank you

Questions?