Wisconsin State Laboratory of Hygiene

UNIVERSITY OF WISCONSIN–MADISON

2014 Update: STEC Diagnosis and Surveillance in Wisconsin

Mike Rauch
Tim Monson
WI State Laboratory of Hygiene
Communicable Disease Division

WCLN Teleconference
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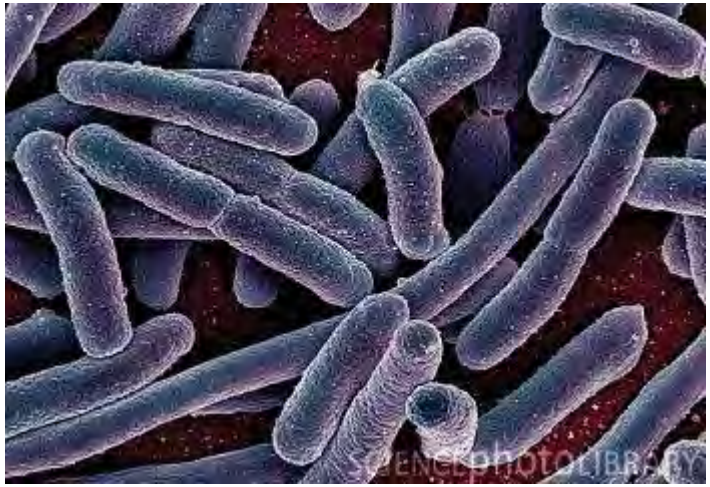
Objectives

- Describe the history of Shiga toxin-producing *E. coli* (STEC) in Wisconsin
- Discuss available diagnostic tests for the detection of Shiga toxins in clinical specimens
- Gain an appreciation for the significance of STEC disease in WI and nationally
- Understand testing that is performed at WSLH and CDC on STEC specimens submitted by clinical health systems

Objectives

- Become aware of ongoing developments in STEC diagnostic testing (Culture-independent diagnostic testing- CIDT) and their potential effects on both clinical and public health laboratories and officials
- Understand the importance of the partnership between clinical, public and environmental health for effective disease surveillance and prevention

Shiga toxin-producing *Escherchia coli* (STEC)



What is STEC?

- Shiga toxin-producing *E. coli* (STEC)—STEC may also be referred to as Verocytotoxin-producing *E. coli* (VTEC) or enterohemorrhagic *E. coli* (EHEC). This pathotype is the one most commonly heard about in the news in association with foodborne outbreaks.

Pathotypes of *E. coli*

- enterotoxigenic *E. coli* (ETEC)
- enteropathogenic *E. coli* (EPEC)
- enteroinvasive *E. coli* (EIEC)
- enteroaggregative *E. coli* (EAEC)
- diffusely adherent *E. coli* (DAEC)
- enterhemorrhagic *E. coli* (EHEC)

Serogroups

Table 1. Laboratory-confirmed STEC infections reported to CDC, with the 20 most frequently reported serogroups listed individually, United States, 2011

Rank	Serogroup	Number Reported	Percent
1	O157	2366	41.1
2	O26	611	10.6
3	O103	548	9.5
4	O111	322	5.6
5	O121	176	3.1
6	O45	135	2.3
7	O145	74	1.3
8	O118	47	0.8
9	O69	22	0.4
10	O76	14	0.2
11	O91	14	0.2
12	O5	9	0.2
13	O153	8	0.1
14	O104	7	0.1
15	O113	6	0.1
16	O165	6	0.1
17	O174	6	0.1
18	O71	6	0.1
19	O146	5	0.1
20	O80	5	0.1
	Sub Total	**4387**	**76.1**
	All other non-O157 STEC	89	1.5
	Unknown*	1200	20.8
	Rough	79	1.4
	Undetermined	8	0.1
	Sub Total	**1376**	**23.9**
		5763	**100**

Virulence Factors

- Two distinct Shigatoxins (Stx1 and Stx2)
- Shigella dysenteriae 1
- Range in virulence
- Additional virulence factors.
  - *eae* gene for intimin
  - ehxA gene for plasmid encoded hemolysin

Virulence Factors

Table 3. Presence of Virulence Genes in Shiga Toxin–Producing *Escherichia coli* Isolates From Patients With Postdiarrheal Hemolytic Uremic Syndrome by Serogroup, 2000-2010

Serogroup	Shiga Toxin			*eae*[a]	*ehxA*[a]
	*stx*1 Alone	*stx*2 Alone	*stx*1 and *stx*2		
O157 (n = 103)[b]	0/103	78/103 (75.7)	25/103 (24.3)		
O111 (n = 4)[c]	0/4	0/4	4/4 (100)	4/4 (100)	4/4 (100)
O121 (n = 2)[c]	0/2	2/2 (100)	0/2	2/2 (100)	2/2 (100)
O145 (n = 5)	0/5	5/5 (100)	0/5	5/5 (100)	5/5 (100)
O26 (n = 2)[d]	1/2 (50.0)	0/2	1/2 (50.0)	2/2 (100)	1/2 (50.0)
O103 (n = 1)	1/1 (100)	0/1	0/1	1/1 (100)	1/1 (100)
O130 (n = 1)	0/1	1/1 (100)	0/1	0/1	1/1 (100)
Unknown (n = 1)[c]	0/1	0/1	1/1 (100)		
Total	**2/119** (1.7)	**86/119** (72.3)	**31/119** (26.1)	**14/15** (93.3)	**14/15** (93.3)

[a] *eae* encodes intimin, and *ehxA* encodes enterohemolysin; *eae* and *ehxA* data were not available for STEC O157 (test not generally performed because genes almost invariably present) or for isolates of unknown serogroup.
[b] Shiga toxin data collected for 103 of 135 STEC O157 isolates (76.3%) from 2007 through 2010.
[c] Virulence factor information is not available for 1 isolate from each of the following serogroups: O111, O121, and unknown.
[d] Excluded 1 Stx1-only STEC O26 isolate from a patient whose stool sample also yielded a *stx* 2-only STEC O157 isolate.

From: Infections in Pediatric Postdiarrheal Hemolytic Uremic Syndrome: Factors Associated With Identifying Shiga Toxin–Producing Escherichia coli

Arch Pediatr Adolesc Med. 2012;166(10):902-909. doi:10.1001/archpediatrics.2012.471

STEC- Background Information

- CDC estimates 19% of O157 STEC and 9% of non-O157 STEC are outbreak related (majority of cases sporadic)
- STEC are low-infectious dose organisms (10-100 cells)
- STEC virulence dependent upon which virulence factors are present in a given strain (Stx1/Stx2, eae, Ehly); evidence suggests Stx and eae are most significant predictors of serious illness

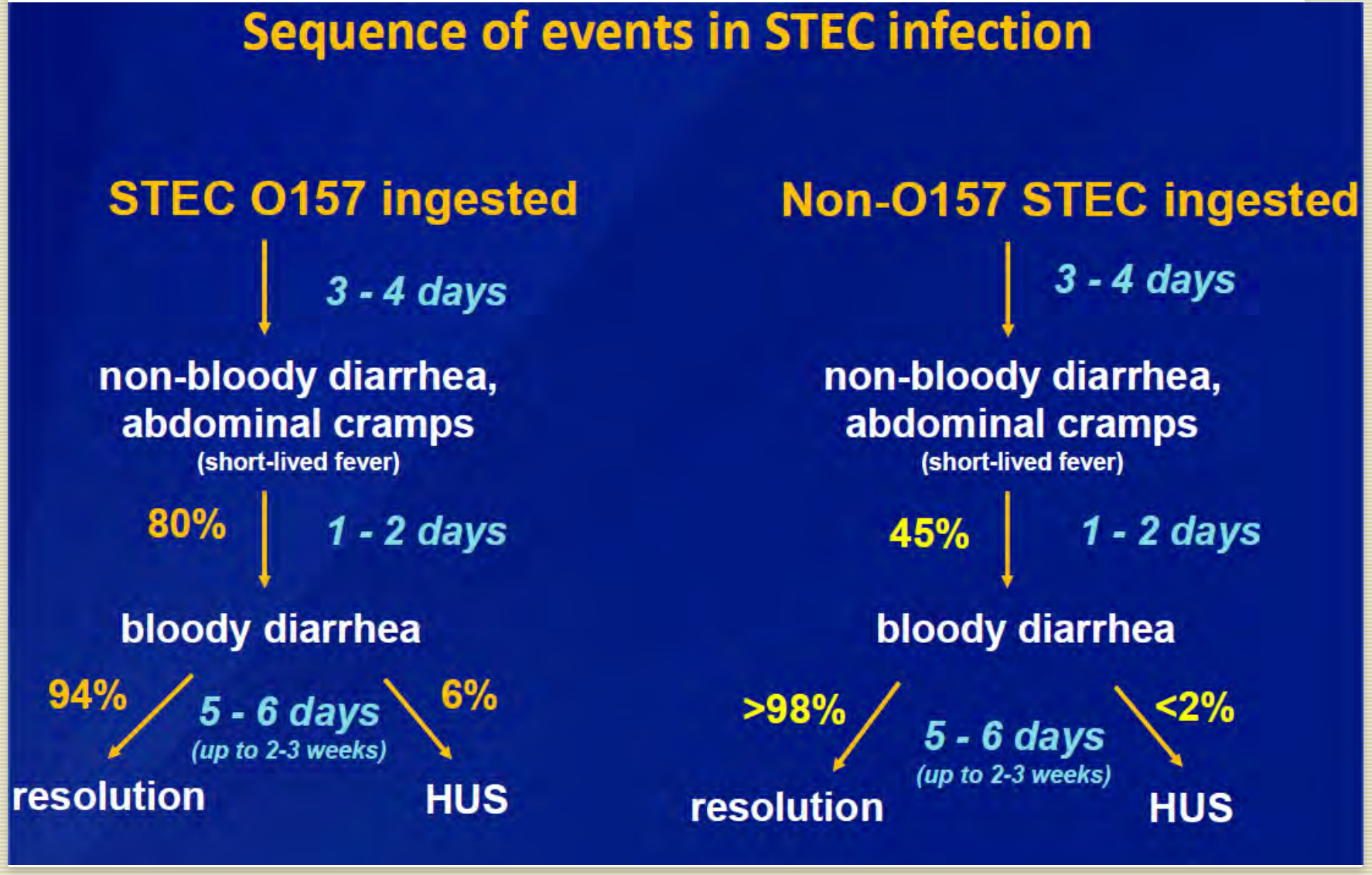
Common Symptoms of STEC Disease

- Diarrhea (sometimes bloody)
- Severe stomach cramps
- Vomiting
- Fever less than 101°F

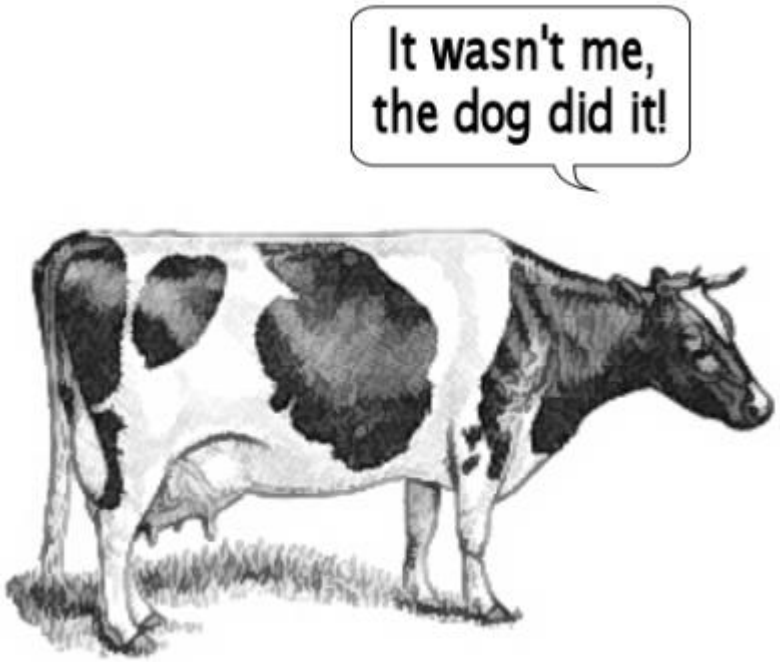
STEC Complications

- Hemolytic Uremic Syndrome (HUS)
  - Occur in about 5–10% of cases
  - Usually STEC O157
  - Renal failure
  - Hemolytic anemia
  - Thrombocytopenia
  - Most recover within a few weeks, but some suffer permanent damage or die

Sequence of events in STEC infection



Where does STEC come from?

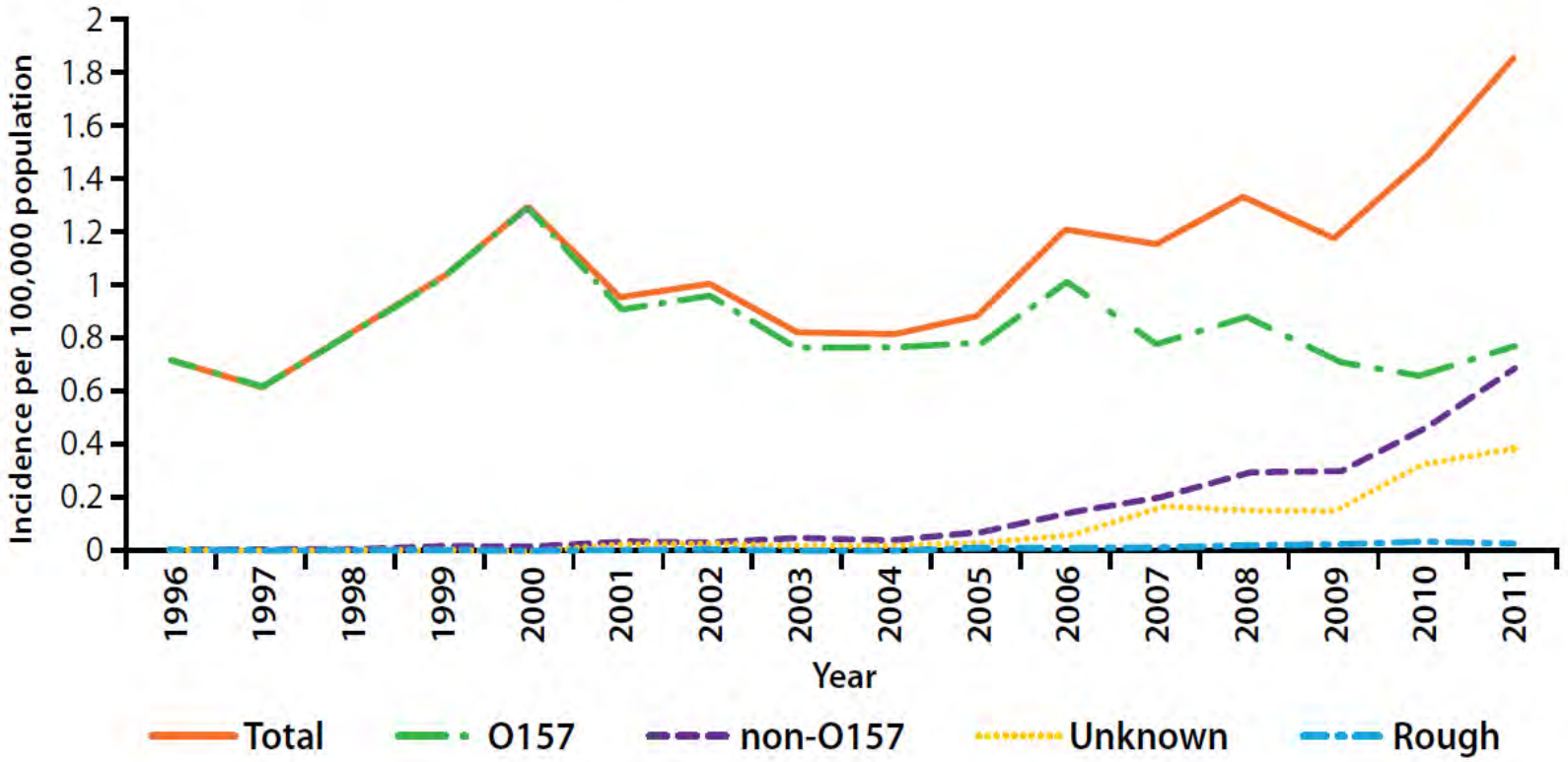


Incidence

- 265,000 Illnesses annually in U.S.
- 3,600 plus hospitalizations
- 30 deaths
- 96,534 STEC O157
- 168,698 non-O157

Incidence

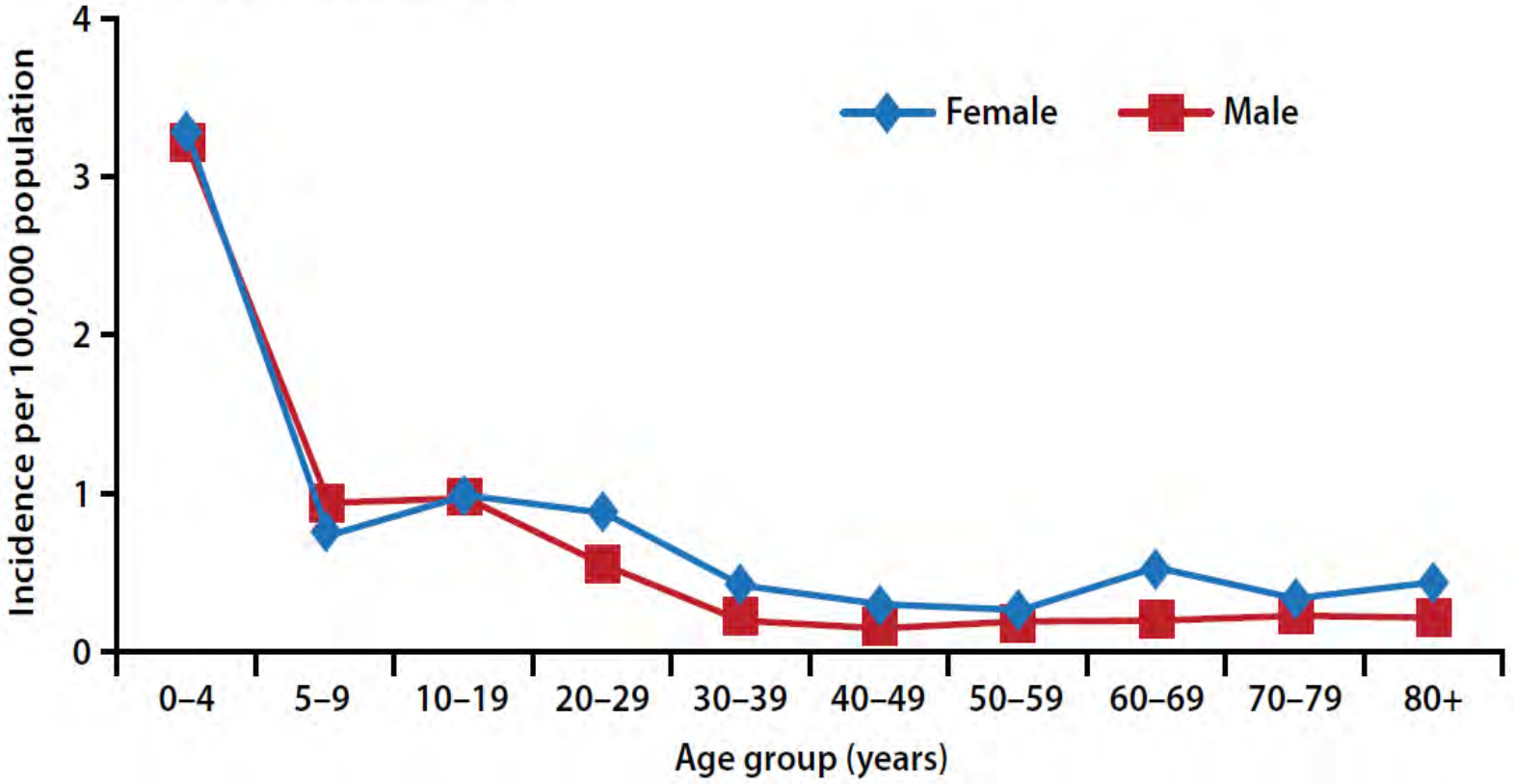
Figure 1. Incidence rate of laboratory-confirmed human STEC infection reported to CDC per 100,000 population, by serogroup and year, United States, 1996–2011 (n=50,158)



Centers for Disease Control and Prevention (CDC). National Shiga toxin-producing Escherichia coli (STEC) Surveillance Annual Report, 2011. Atlanta, Georgia: US Department of Health and Human Services, CDC, 2013.

Incidence

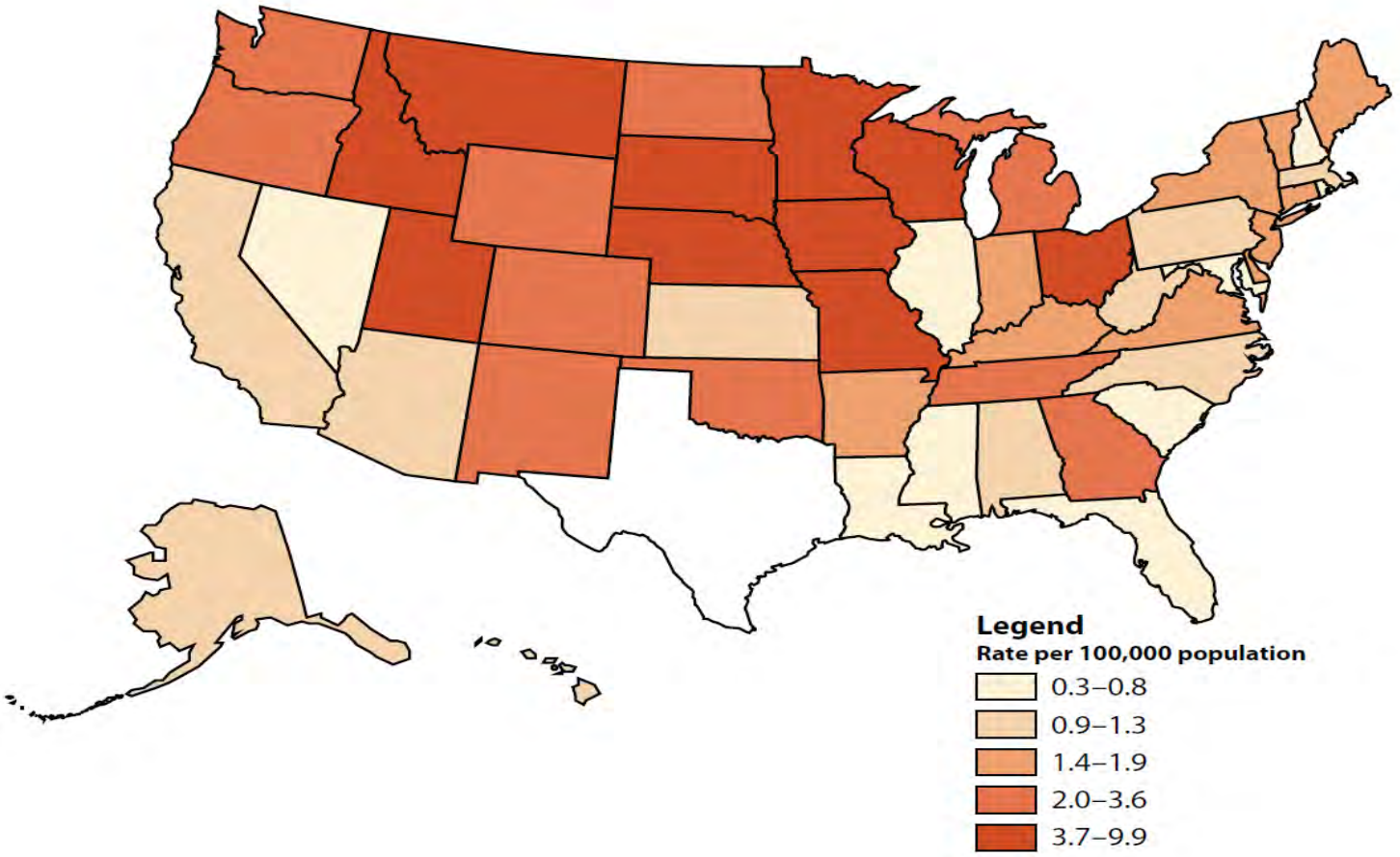
Figure 3b. Incidence rate of laboratory-confirmed non-O157 STEC infection reported to CDC, by age group and sex, United States, 2011 (n=2,014)



Centers for Disease Control and Prevention (CDC). National Shiga toxin-producing Escherichia coli (STEC) Surveillance Annual Report, 2011. Atlanta, Georgia: US Department of Health and Human Services, CDC, 2013.

STEC

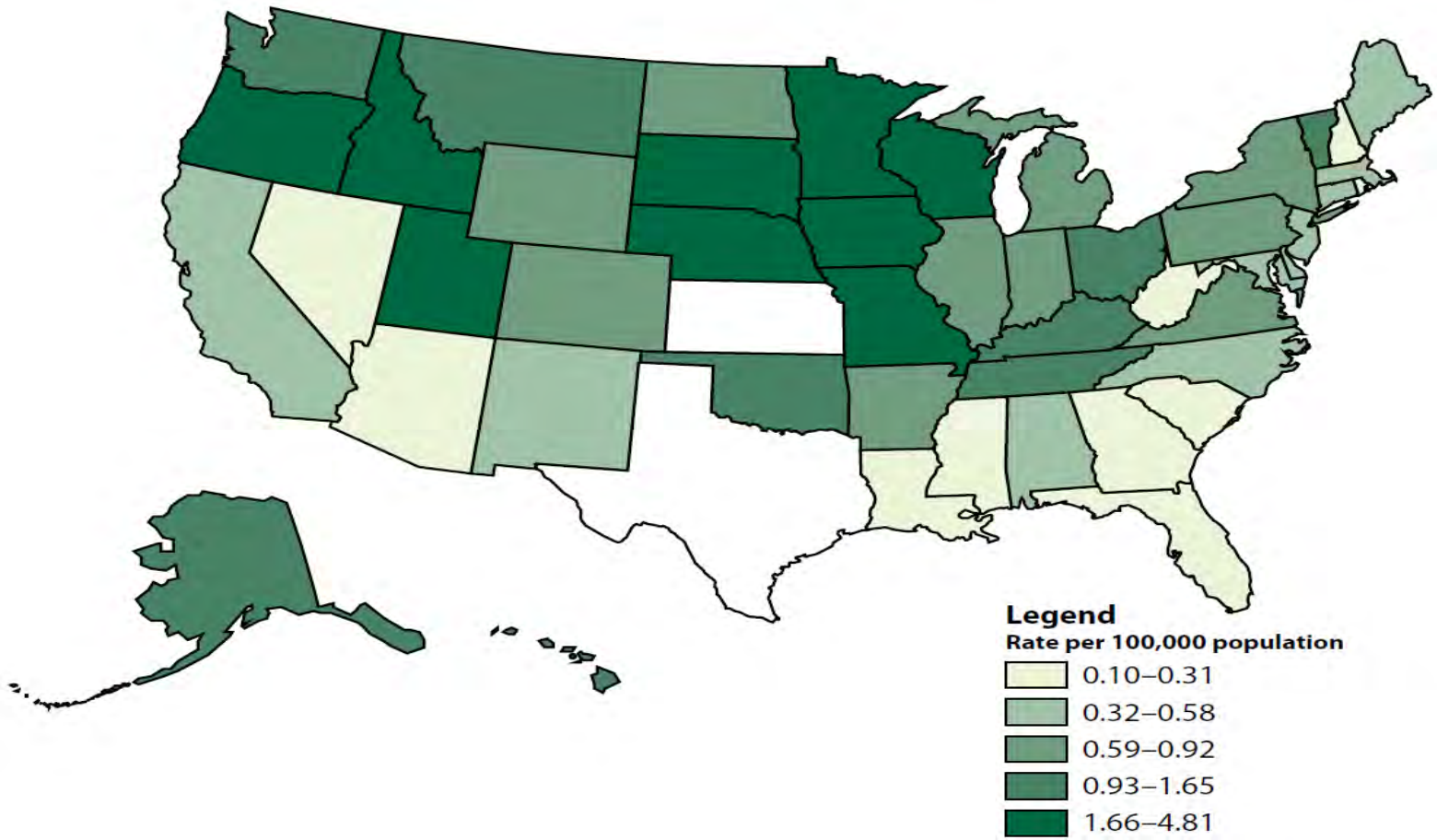
Figure 2a. Incidence rate of laboratory-confirmed STEC infection reported to CDC, by state, United States, 2011 (n=5,763)*



Centers for Disease Control and Prevention (CDC). National Shiga toxin-producing Escherichia coli (STEC) Surveillance Annual Report, 2011. Atlanta, Georgia: US Department of Health and Human Services, CDC, 2013.

STEC O157

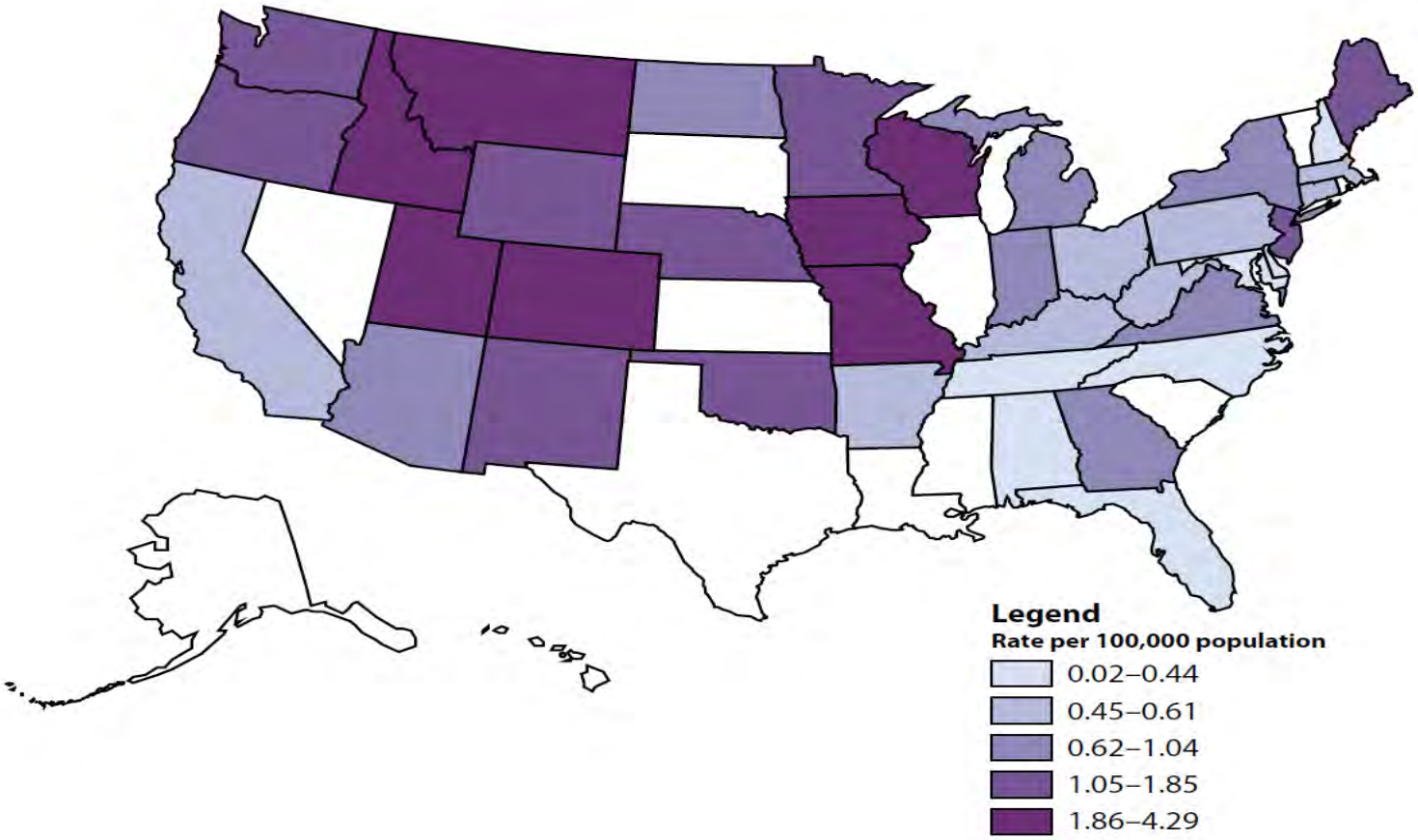
Figure 2b. Incidence rate of laboratory-confirmed STEC O157 infection reported to CDC, by state, United States, 2011 (n=2,366)*



Centers for Disease Control and Prevention (CDC). National Shiga toxin-producing Escherichia coli (STEC) Surveillance Annual Report, 2011. Atlanta, Georgia: US Department of Health and Human Services, CDC, 2013.

Non-O157 STEC

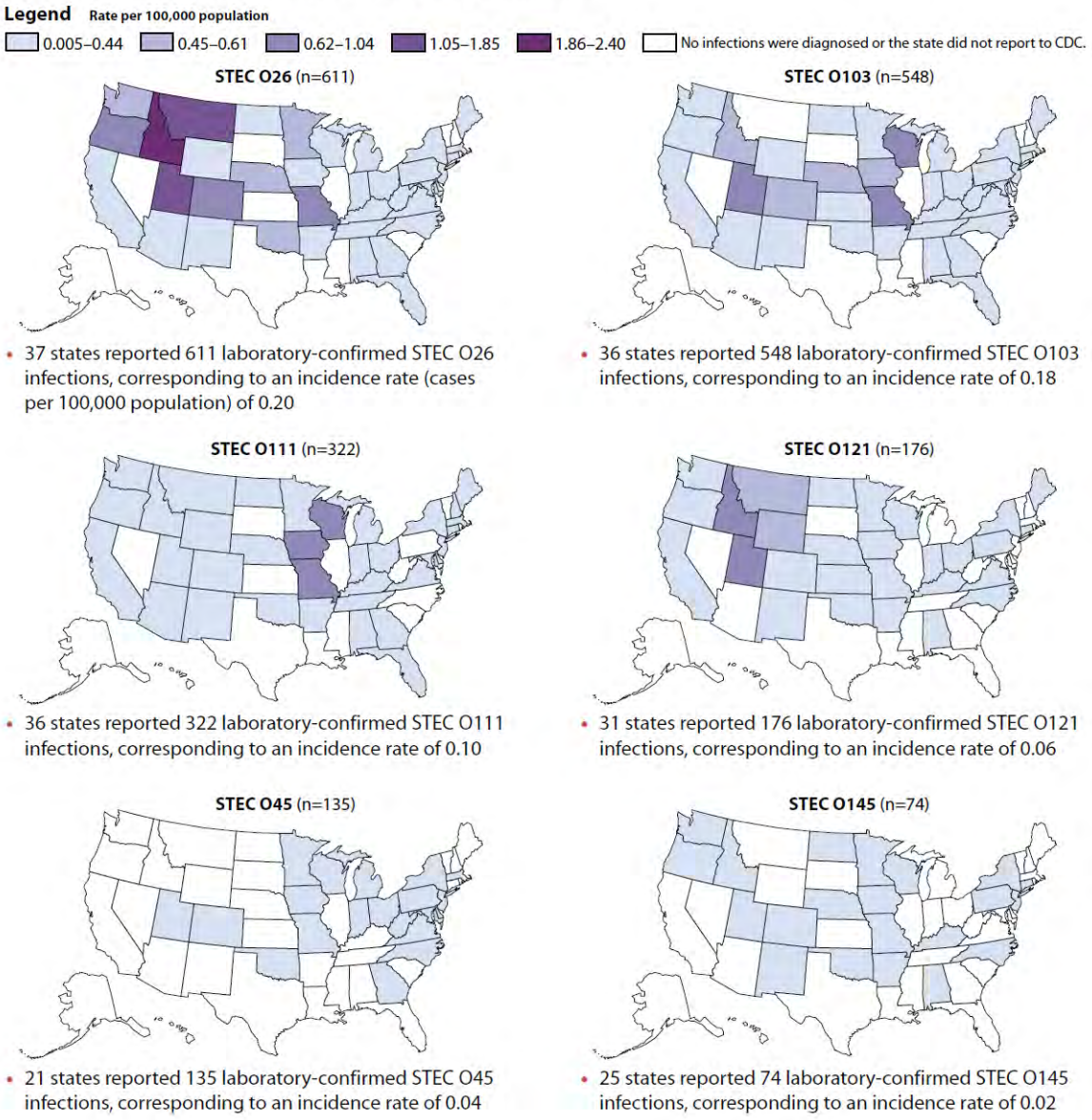
Figure 2c. Incidence rate of laboratory-confirmed non-O157 STEC infection reported to CDC, by state, United States, 2011 (n=2,118)*



Centers for Disease Control and Prevention (CDC). National Shiga toxin-producing Escherichia coli (STEC) Surveillance Annual Report, 2011. Atlanta, Georgia: US Department of Health and Human Services, CDC, 2013.

The "Big Six"

Figure 2d. Incidence rate of laboratory-confirmed non-O157 STEC infection, top 6 non-O157 STEC serogroups, reported to CDC by state, United States, 2011 (n=1,866)



- 37 states reported 611 laboratory-confirmed STEC O26 infections, corresponding to an incidence rate (cases per 100,000 population) of 0.20
- 36 states reported 548 laboratory-confirmed STEC O103 infections, corresponding to an incidence rate of 0.18
- 36 states reported 322 laboratory-confirmed STEC O111 infections, corresponding to an incidence rate of 0.10
- 31 states reported 176 laboratory-confirmed STEC O121 infections, corresponding to an incidence rate of 0.06
- 21 states reported 135 laboratory-confirmed STEC O45 infections, corresponding to an incidence rate of 0.04
- 25 states reported 74 laboratory-confirmed STEC O145 infections, corresponding to an incidence rate of 0.02

Centers for Disease Control and Prevention (CDC). National Shiga toxin-producing Escherichia coli (STEC) Surveillance Annual Report, 2011. Atlanta, Georgia: US Department of Health and Human Services, CDC, 2013.

What About Wisconsin?

2010 – 2013 STEC Confirmed Cases (n=1013)

O157 vs. NonO157

- O157 – 492
- NonO157 – 521

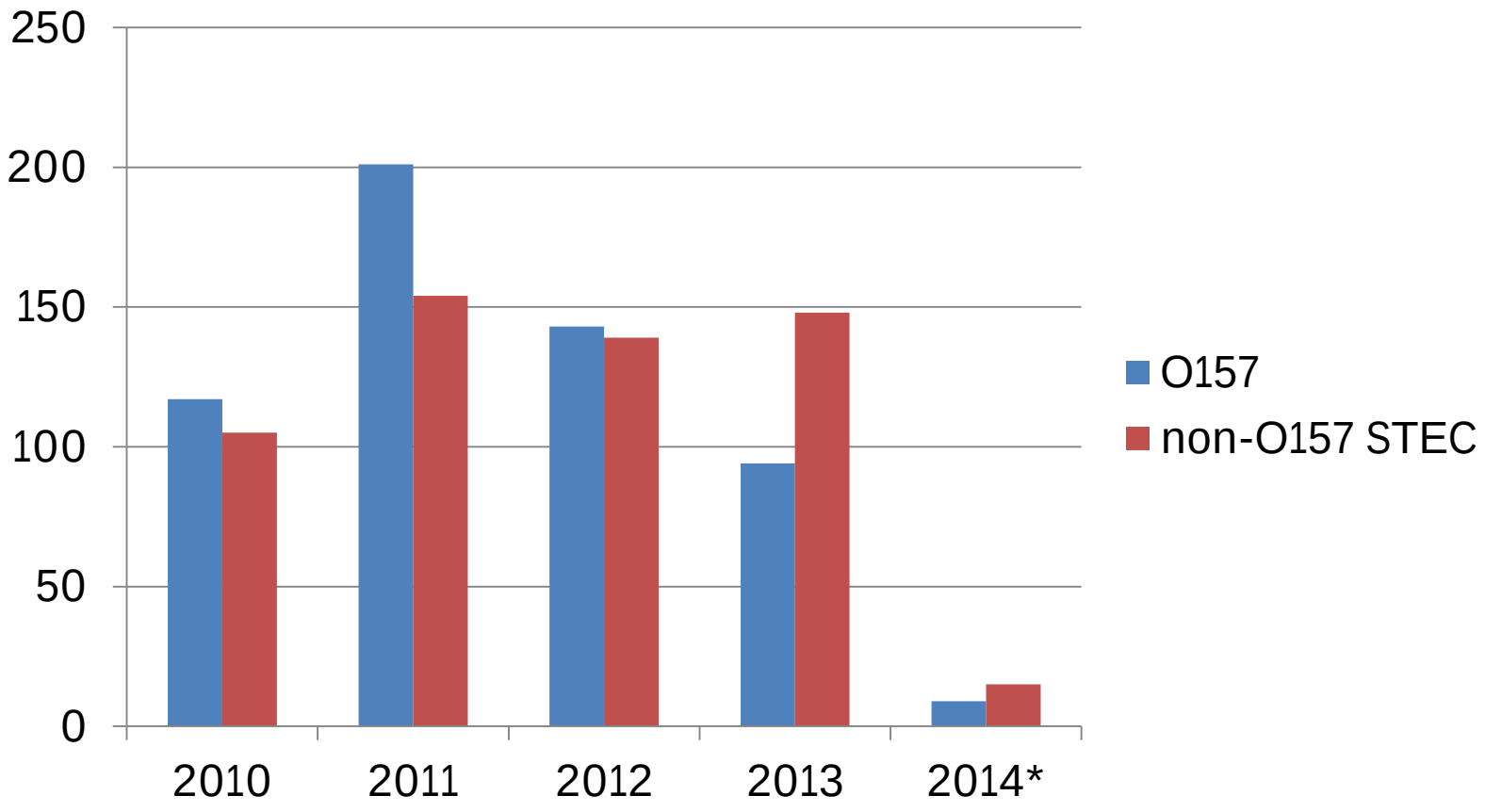
Big 7 vs. NonO157

- Big 7 – 935
- NonBig7 – 78

O group	Number
O157	492
O111	127
O103	140
O26	73
O45	57
O145	17
O121	29
Other	78 (none identified >4 times)

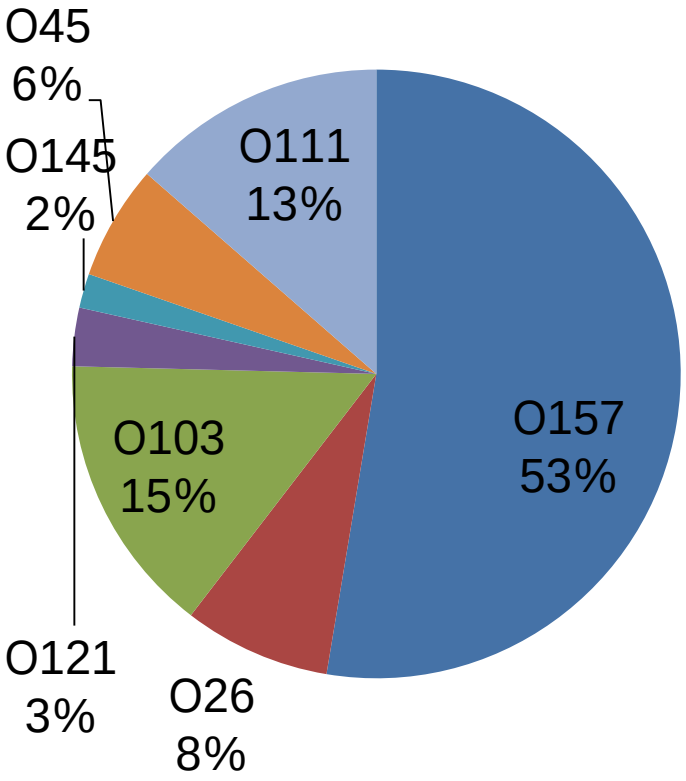
O157 vs. non-O157 STEC

* as of 3/11/2014

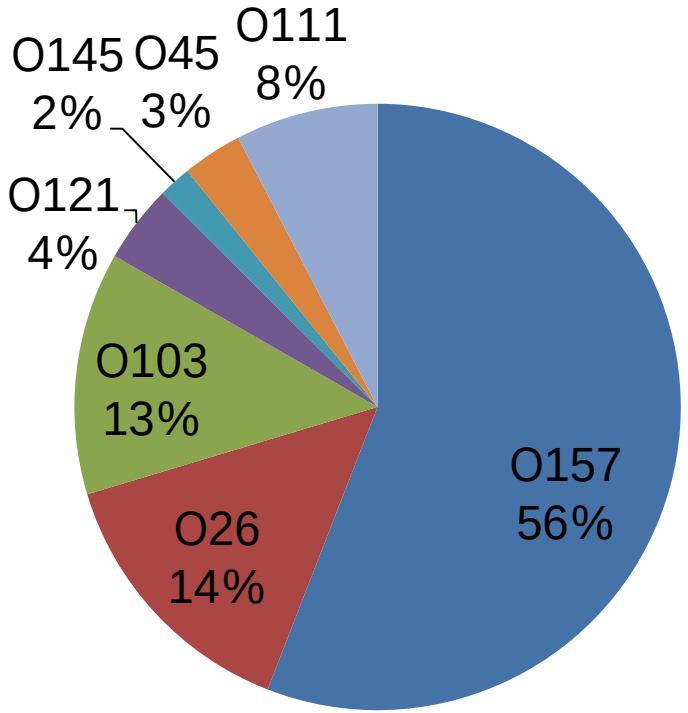


O157 vs. non-O157 STEC

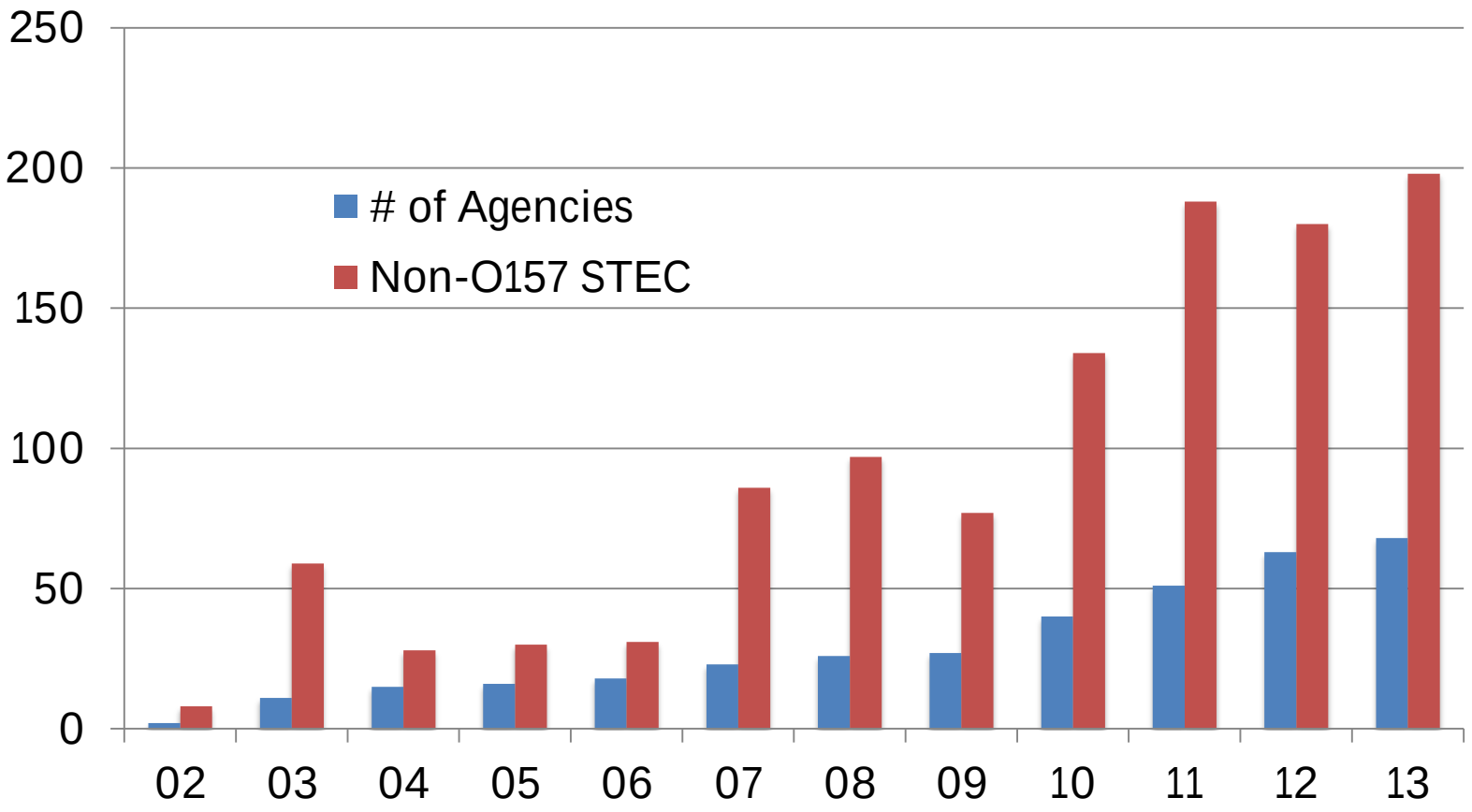
WI 2010 - 2013



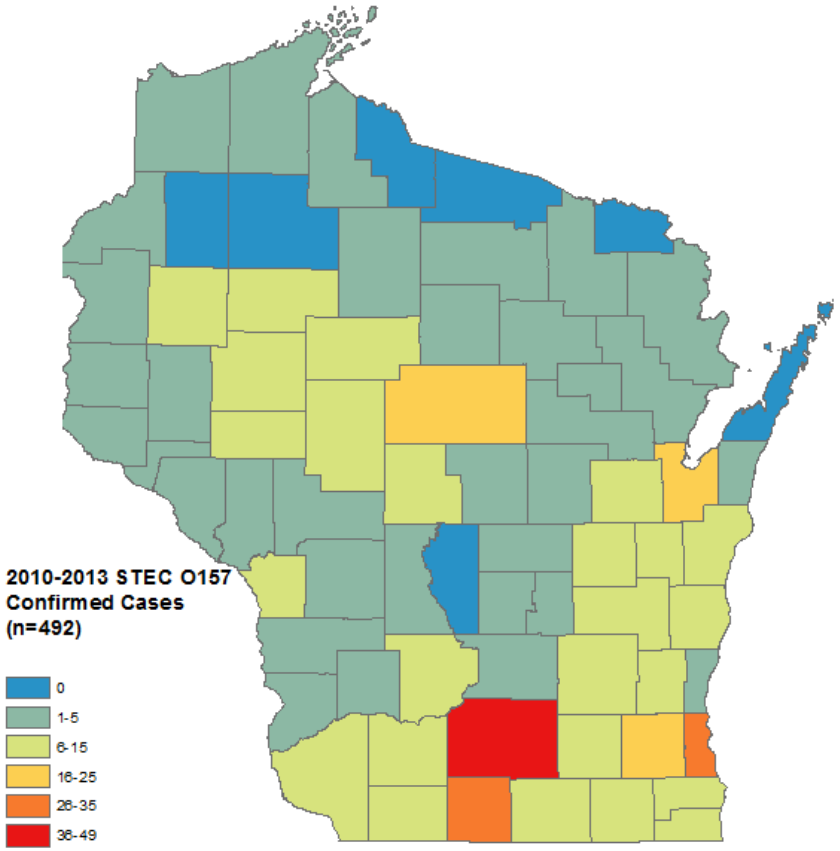
2011 National LEDS



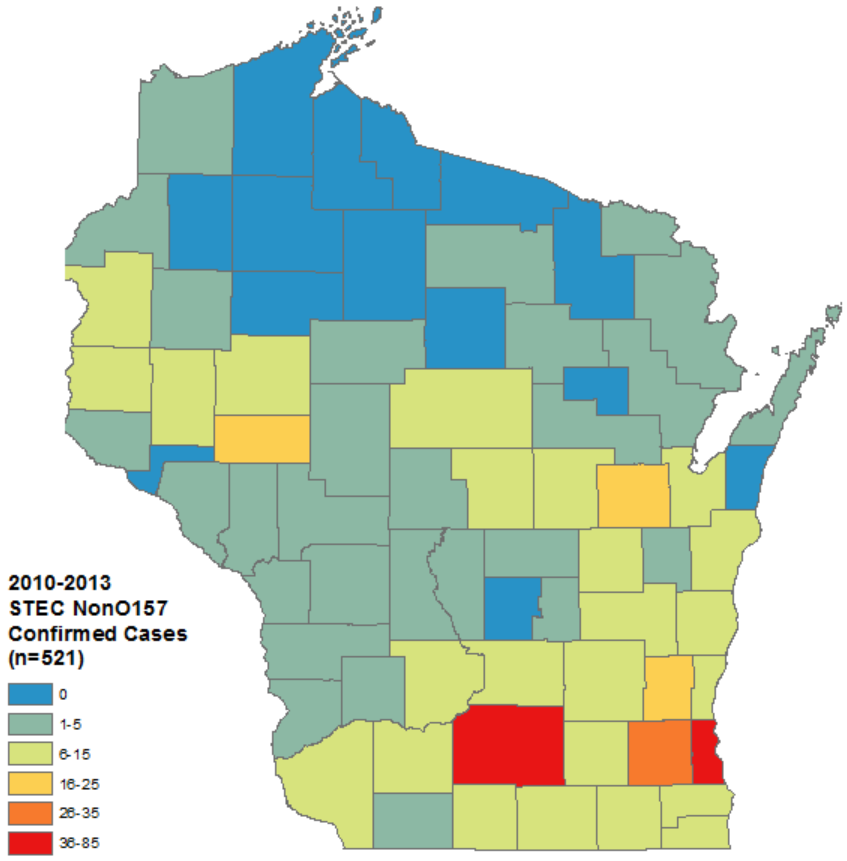
Agencies & STEC Isolated



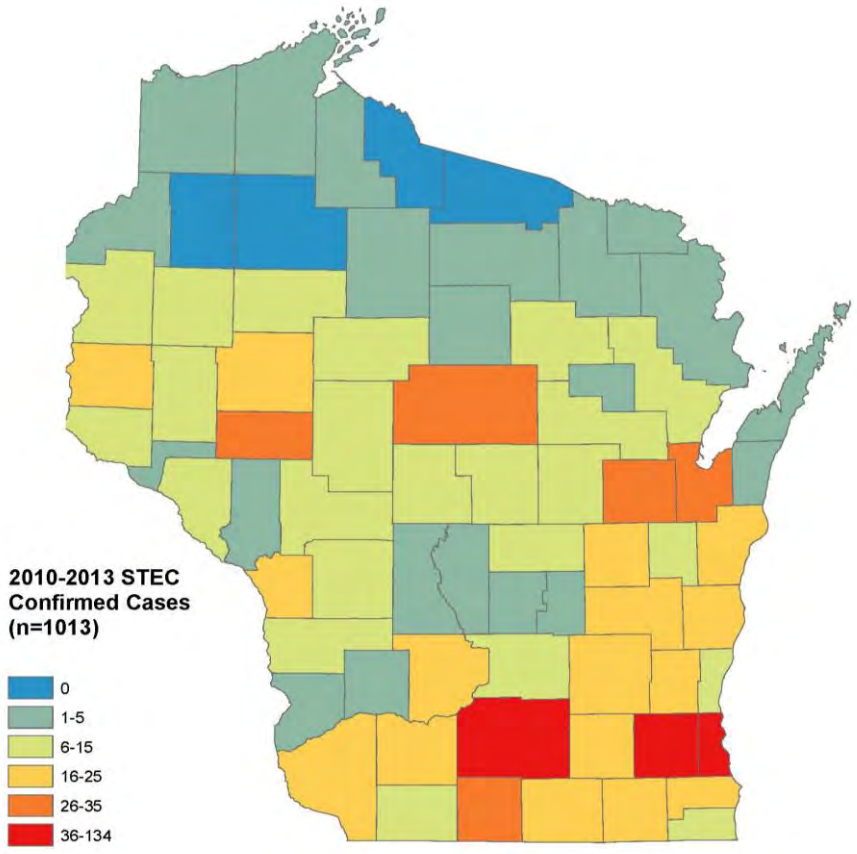
2010-13 O157 STEC



2010-13 non-O157 STEC



2010-13 Combined STEC



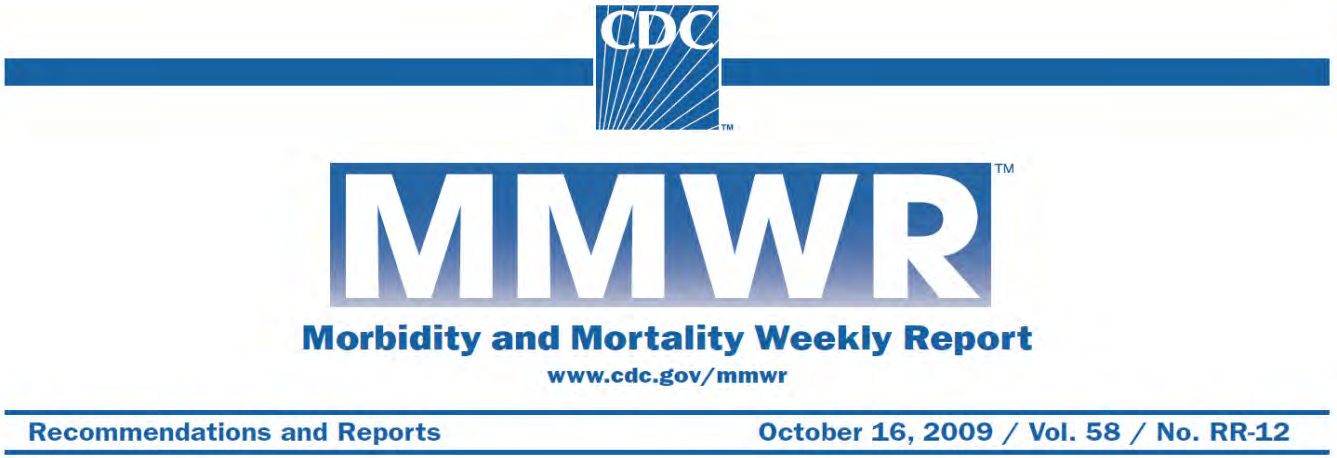
Wisconsin Case Definition:

- **Confirmed:** A case that meets the laboratory criteria for confirmation. When available, O and H antigen serotype characterization should be reported
- **Probable:** A case with isolation of *E. coli* O157 from a clinical specimen, without confirmation of H antigen or Shiga toxin production, OR a clinically compatible case that is epidemiologically linked to a confirmed or probable case, OR identification of an elevated antibody titer to a known Shiga toxin-producing *E. coli* serotype from a clinically compatible case
- **Suspected:** Identification of Shiga toxin in a specimen from a clinically compatible case without the isolation of the Shiga toxin-producing E. coli

Laboratory Criteria for Confirmation:

- Isolation of Shiga toxin-producing Escherichia coli from a clinical specimen.
- E. coli O157:H7 isolates may be assumed to be Shiga toxin-producing.
- For all other E. coli isolates, Shiga toxin production or the presence of Shiga toxin genes must be confirmed to be considered STEC. Examples of assays used to detect Shiga toxin production include Shiga toxin EIA or PCR.

http://www.cdc.gov/mmwr/PDF/rr/rr5812.pdf



Guidelines for the Clinical Laboratory:

- All stools submitted for testing from patients with acute, community-acquired diarrhea should be cultured for O157 STEC on selective and differential media
- Stools from patients with acute, community-acquired diarrhea should be simultaneously tested for non-O157 STEC with a test that detects either Shiga toxins or the genes that encode for these toxins

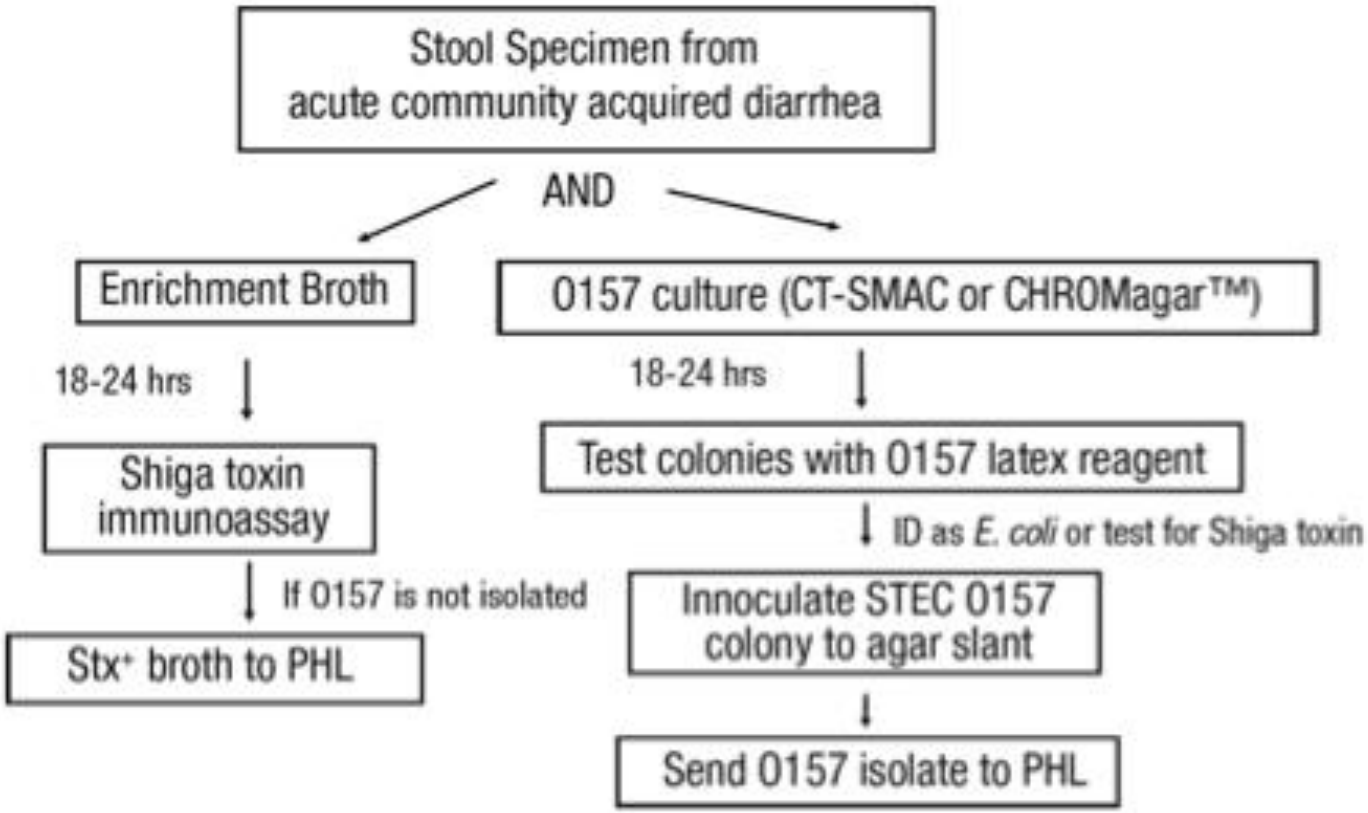
Guidelines for the Clinical Laboratory:

- All O157 STEC isolates should be forwarded as soon as possible to a state or local public health laboratory (PHL) for confirmation and molecular characterization (PFGE, MLVA, virulence genes)
- Detection of STEC or Shiga toxin should be promptly reported to the physician, PHL and proper public health authorities

Guidelines for the Clinical Laboratory:

- Any specimens or enrichment broths in which Shiga toxin or STEC has been detected but from which no O157 STEC isolates are recovered, should be forwarded as soon as possible to a state or local public health laboratory for isolation of the STEC

Clinical Laboratory Recommendations



Justification for STEC Testing

- STEC may be as or more prevalent than other enteric bacterial pathogens routinely tested for:
  - STEC may be found in 0-4% of stools
  - *Salmonella* may be found in 1.9-4.8%
  - *Shigella* may be found in 0.2-3.1%
  - *Campylobacter* may be found in 0.9-9.3%

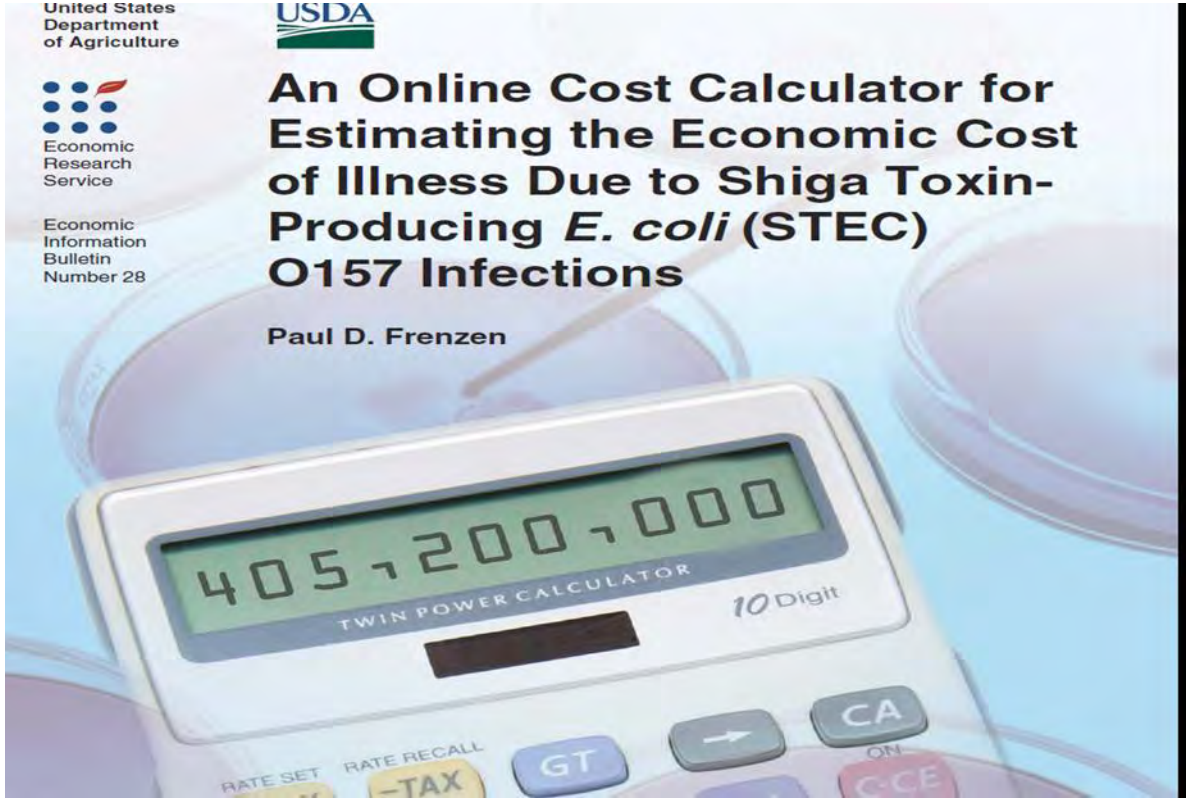
FoodNet Data, CDC

Early HUS Identification and Treatment is Critical

- 15% of culture + *E. coli* O157:H7 patients < 10 yrs of age will develop HUS
- Strong evidence suggesting that STEC isolates that produce Stx2, and in particular Stx2 only, have a higher likelihood to lead to HUS development
- Evidence supporting improved outcomes in individuals that receive prompt intervention (parenteral fluid admin. may prevent renal damage)

Prevention of Illness is Hard to Measure;Hard to Sell

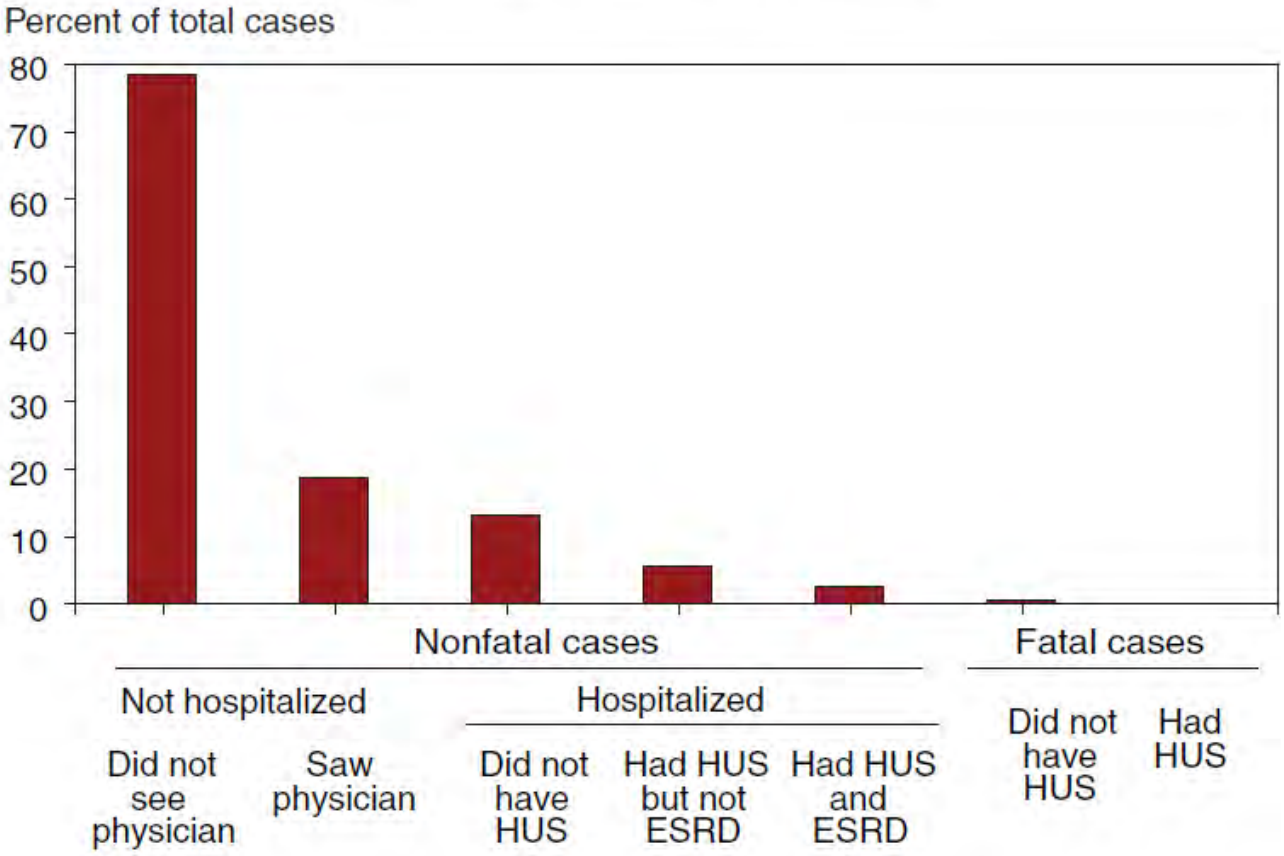
- Even though non-O157 STEC are low prevalence, single case of HUS may cost the healthcare system over $6.1 million



Annual Cases by Severity

Figure 1

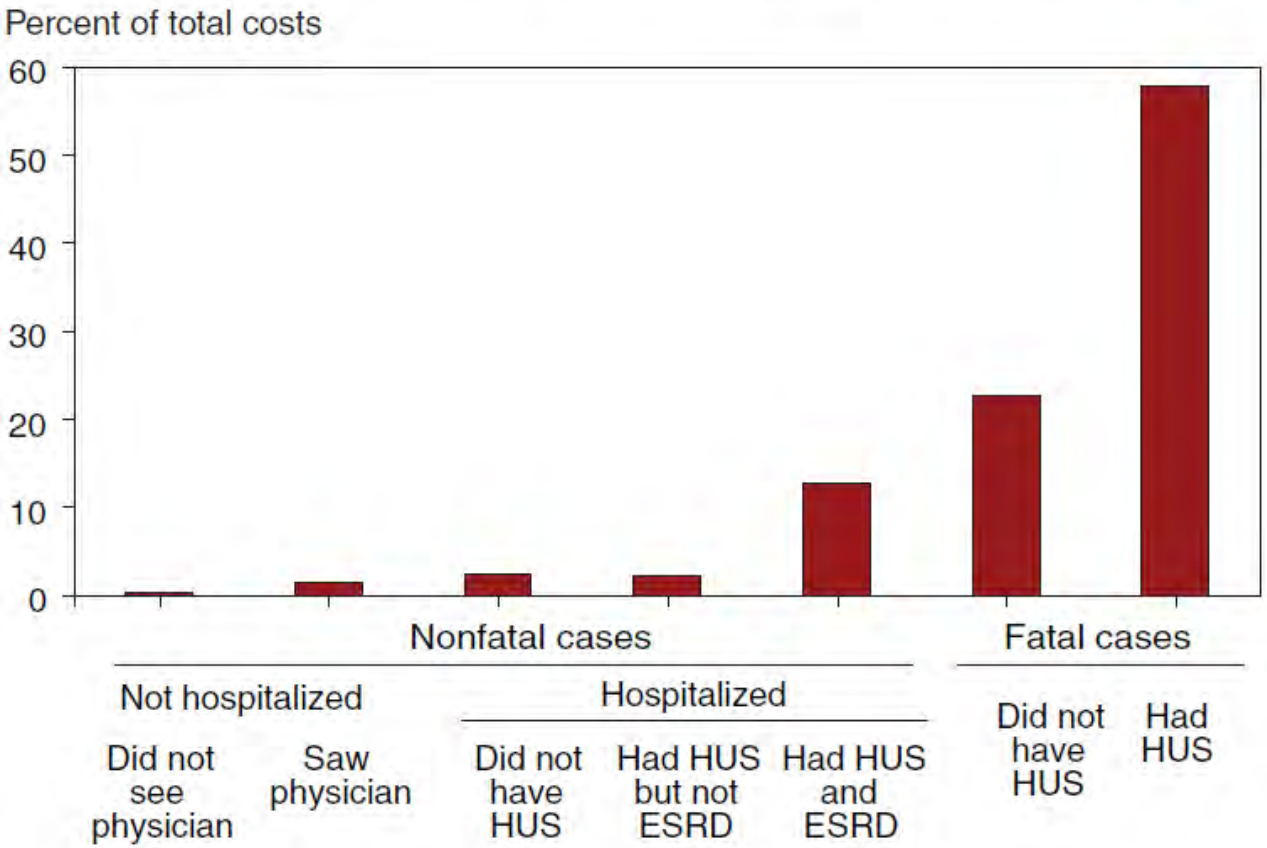
Distribution of annual STEC O157 cases by severity



Source: USDA, ERS estimate based on FoodNet data, reported in Frenzen et al. (2005).

Costs by Severity

Figure 2
Distribution of annual economic costs of STEC O157 by severity



Source: USDA, ERS estimate reported in Frenzen et al. (2005).

Economic Costs

Table 1

Annual economic cost of illness due to Shiga toxin-producing *E. coli* (STEC) O157

Severity category	Estimated annual STEC O157 cases[1]	Estimated annual economic cost[2]	Estimated average cost per case[2]
	Number	*Million dollars*	*Dollars*
Nonfatal cases			
1. Not hospitalized, did not see a physician	57,656	1.5	26
2. Not hospitalized, saw a physician	13,656	6.0	441
3. Hospitalized, did not have HUS	1,797	10.1	5,599
4. Hospitalized, had HUS but not ESRD	300	9.3	30,998
5. Hospitalized, had HUS and ESRD	10	51.7	5,173,594
Fatal cases			
6. Did not have HUS	23	92.0	3,998,265
7. Had HUS	38	234.7	6,175,500
Total	73,480	405.2	5,515

Notes: HUS = hemolytic uremic syndrome. ESRD = end-stage renal disease.
[1]Derived from the CDC estimate of the annual number of STEC O157 cases, hospitalizations, and deaths in 1997. See Frenzen et al. (2005).
[2]2003 dollars. The average cost per case was calculated from unrounded totals.

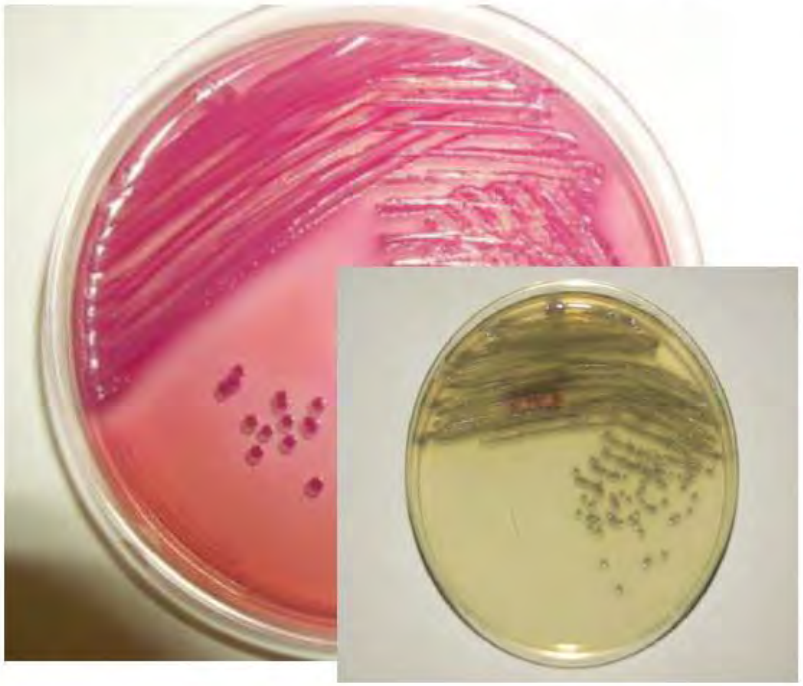
Source: USDA, ERS using data from Frenzen et al. (2005).

Testing at WSLH

WSLH Culture Results

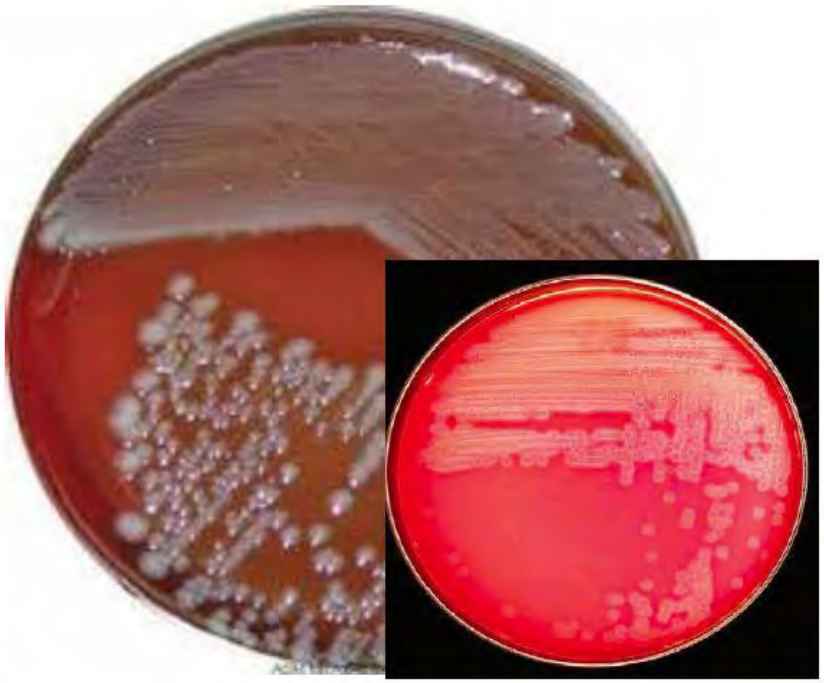
CT-SMAC Plate

(sorbitol fermentation positive; sorbitol negative example inset)

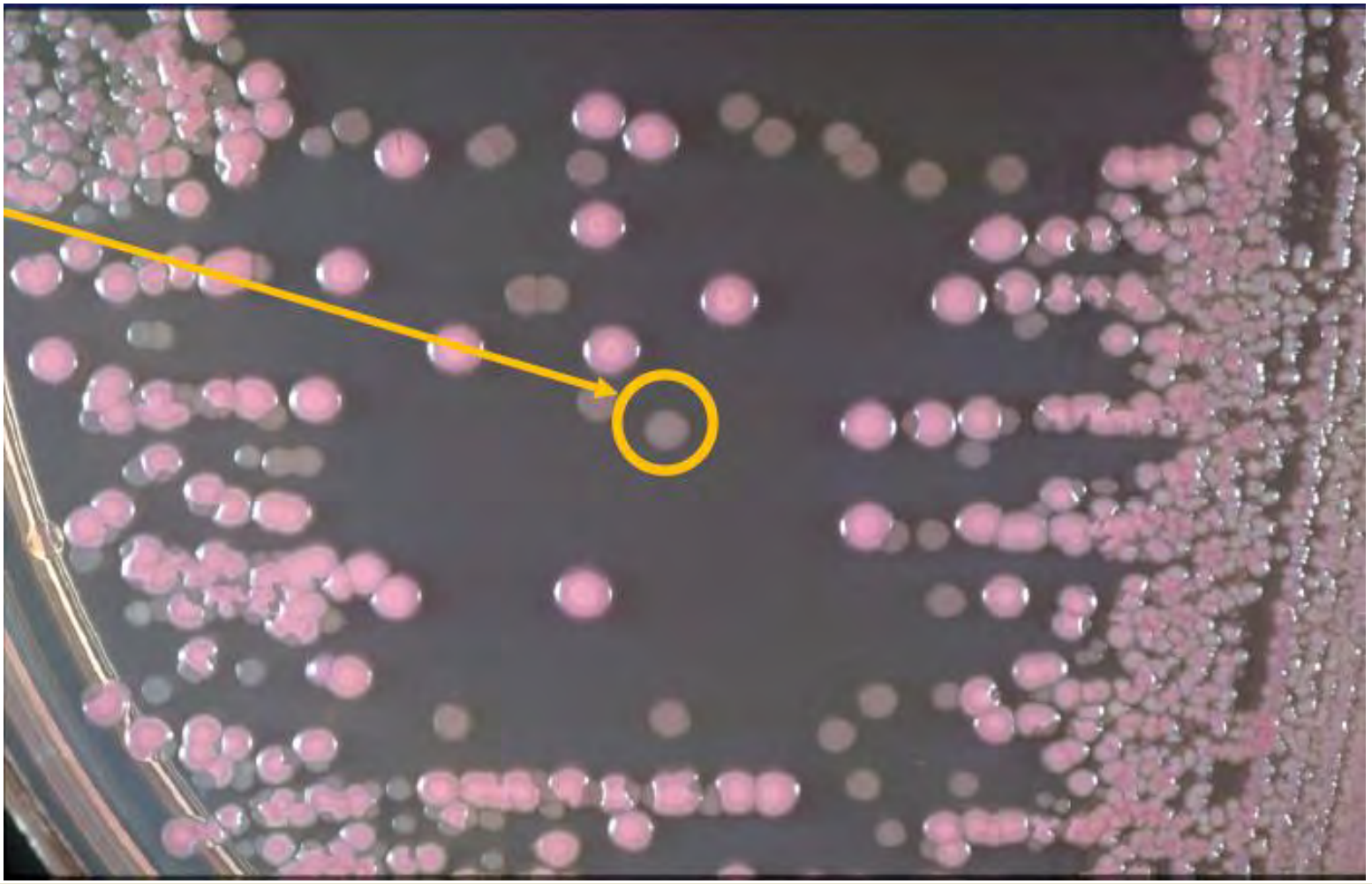


Washed SBA Plate

(non-hemolytic; example of an expected STEC Ehly + result inset)

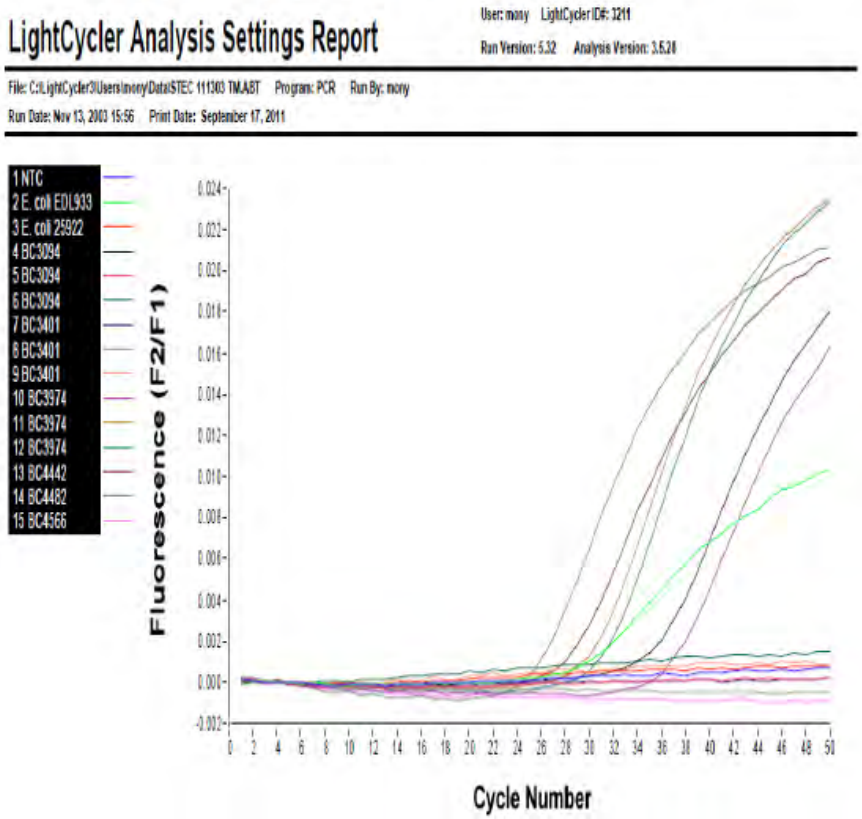


E. Coli O157:H7 on SMAC Plate

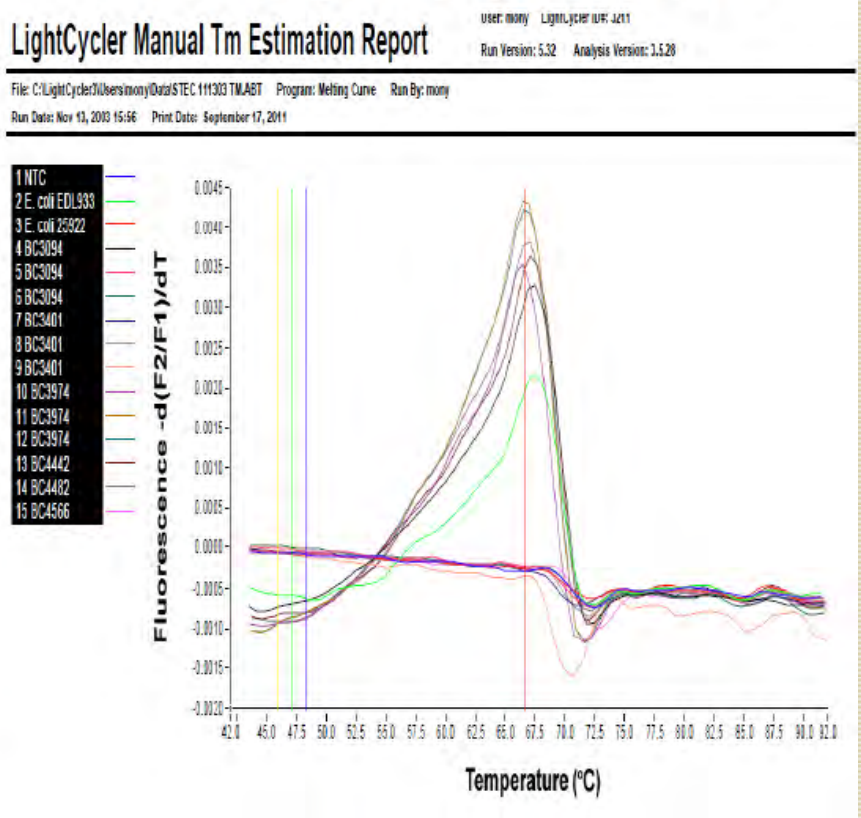


WSLH PCR Results

stx1 and *stx2* detection- fluorescent probes



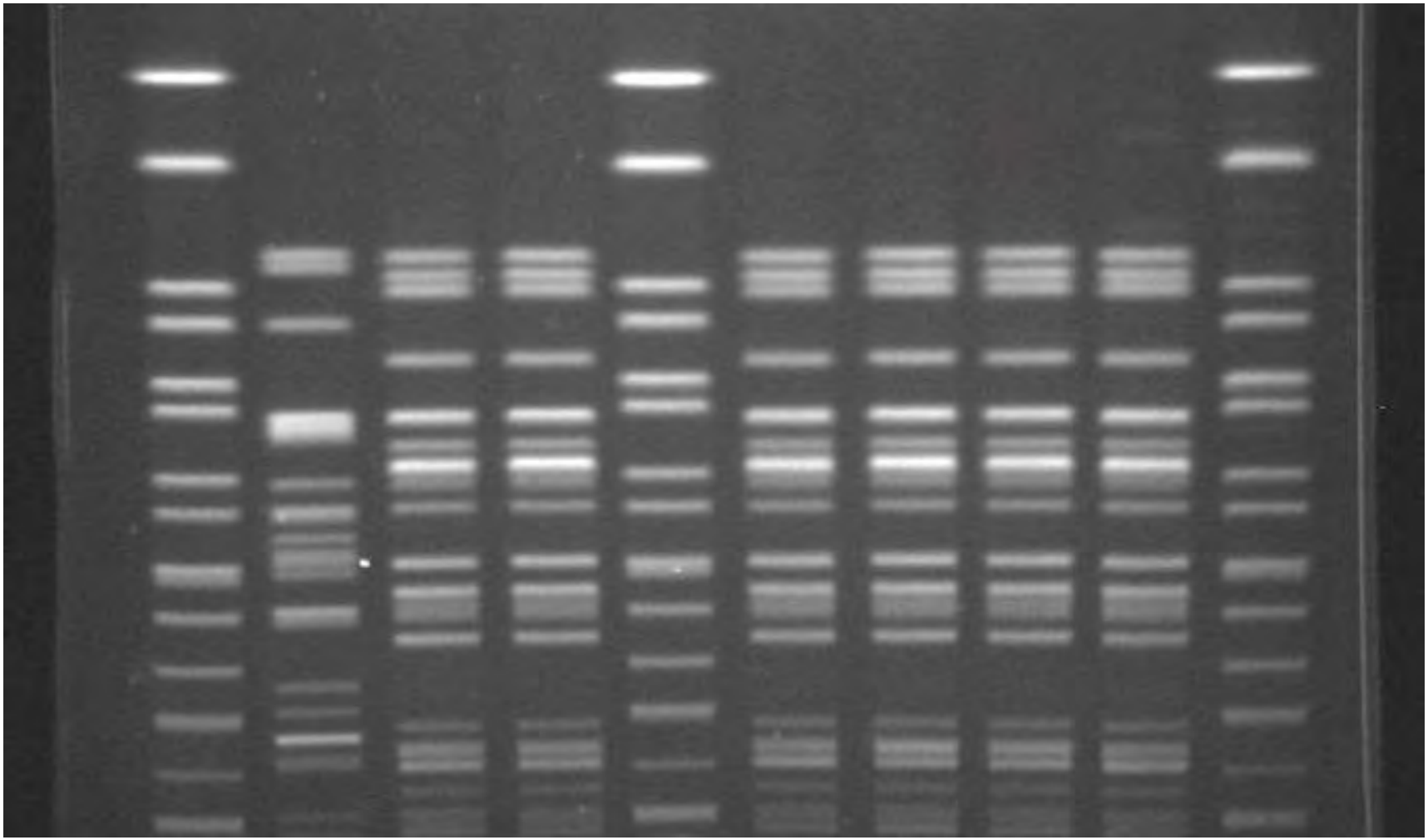
stx1 and *stx2* differentiation by melting temperature



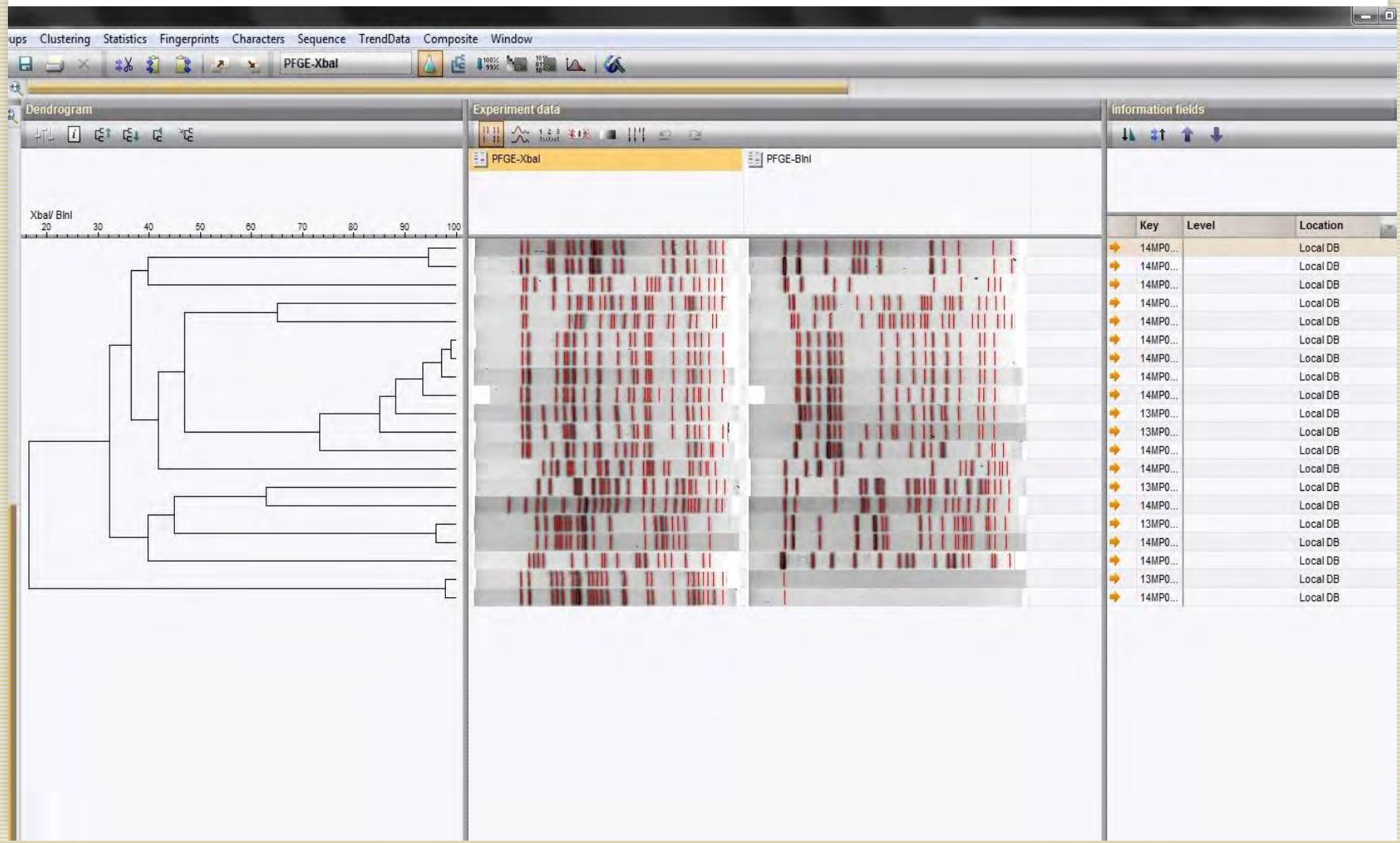
WSLH Serogrouping

- O157 and big six non-O157 by slide agglutination

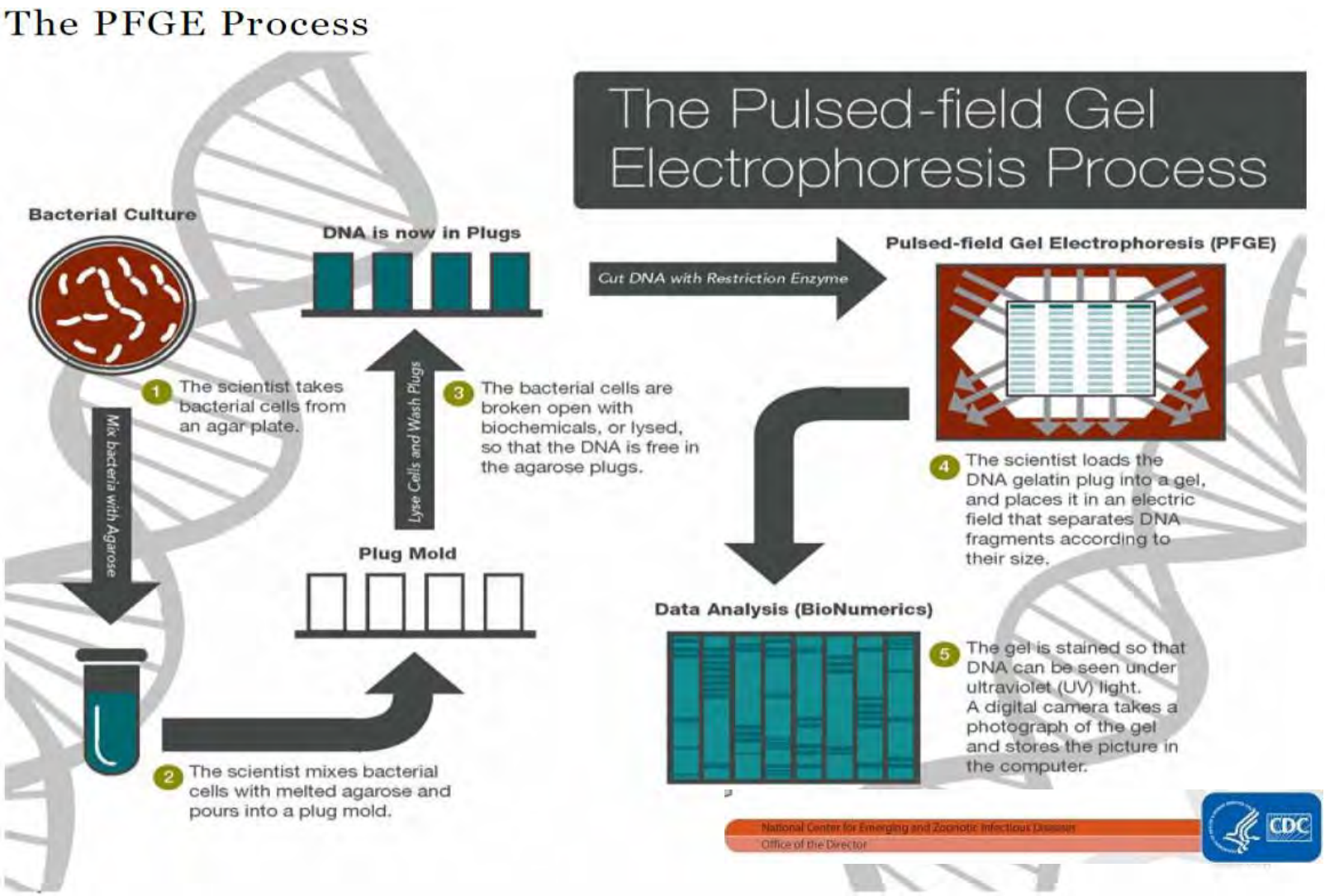
WSLH PFGE Subtyping



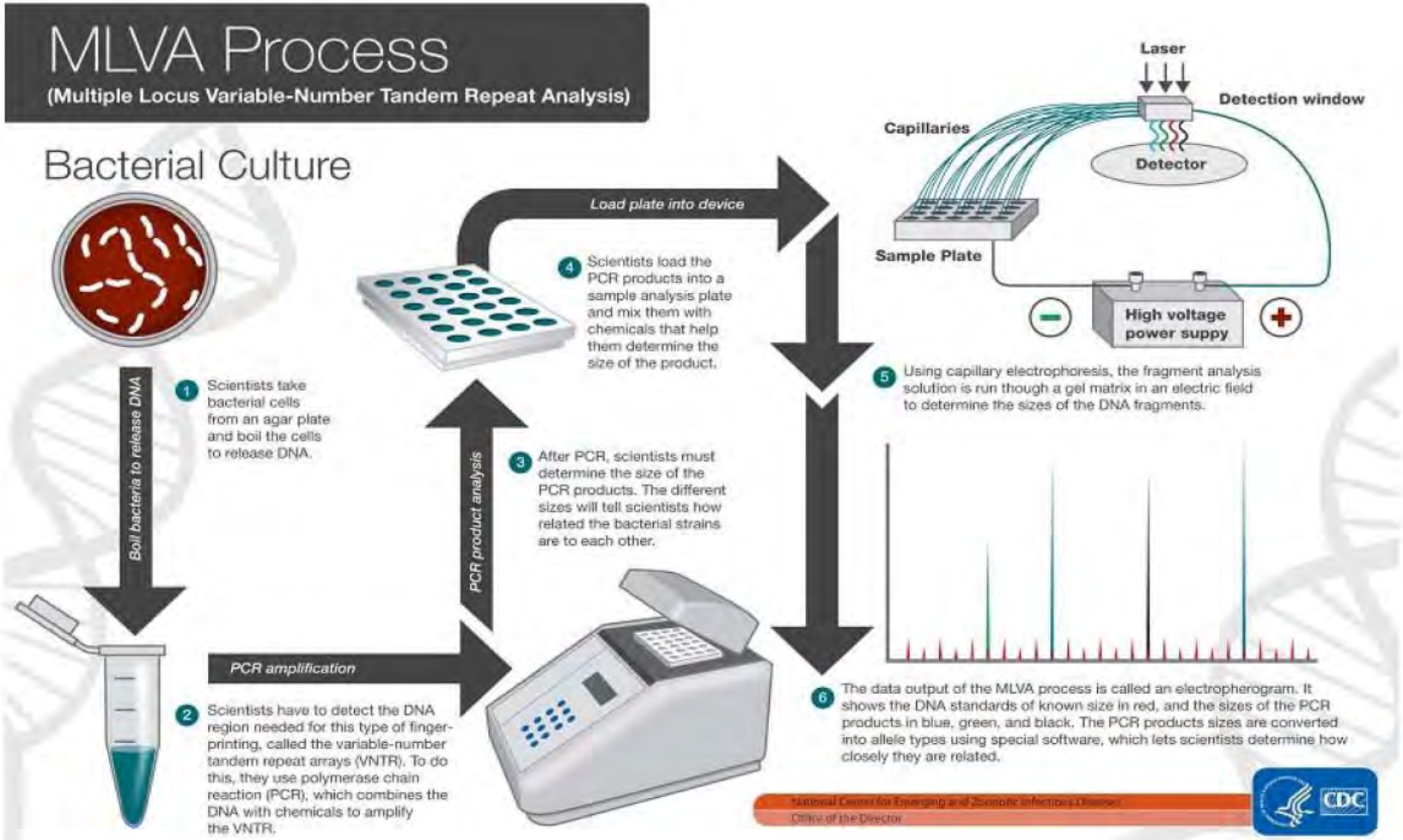
WSLH PFGE Subtyping



PFGE


The PFGE Process
The Pulsed-field Gel Electrophoresis Process
Bacterial Culture
DNA is now in Plugs
Cut DNA with Restriction Enzyme
Pulsed-field Gel Electrophoresis (PFGE)
1 The scientist takes bacterial cells from an agar plate.
Mix bacteria with Agarose
Lyse Cells and Wash Plugs
3 The bacterial cells are broken open with biochemicals, or lysed, so that the DNA is free in the agarose plugs.
4 The scientist loads the DNA gelatin plug into a gel, and places it in an electric field that separates DNA fragments according to their size.
Plug Mold
Data Analysis (BioNumerics)
5 The gel is stained so that DNA can be seen under ultraviolet (UV) light. A digital camera takes a photograph of the gel and stores the picture in the computer.
2 The scientist mixes bacterial cells with melted agarose and pours into a plug mold.
National Center for Emerging and Zoonotic Infectious Diseases
Office of the Director
CDC

MLVA


MLVA Process
(Multiple Locus Variable-Number Tandem Repeat Analysis)
Bacterial Culture
Boil bacteria to release DNA
1 Scientists take bacterial cells from an agar plate and boil the cells to release DNA.
PCR amplification
2 Scientists have to detect the DNA region needed for this type of finger-printing, called the variable-number tandem repeat arrays (VNTR). To do this, they use polymerase chain reaction (PCR), which combines the DNA with chemicals to amplify the VNTR.
PCR product analysis
3 After PCR, scientists must determine the size of the PCR products. The different sizes will tell scientists how related the bacterial strains are to each other.
Load plate into device
4 Scientists load the PCR products into a sample analysis plate and mix them with chemicals that help them determine the size of the product.
Laser
Detection window
Capillaries
Detector
Sample Plate
High voltage power suppy
5 Using capillary electrophoresis, the fragment analysis solution is run though a gel matrix in an electric field to determine the sizes of the DNA fragments.
6 The data output of the MLVA process is called an electropherogram. It shows the DNA standards of known size in red, and the sizes of the PCR products in blue, green, and black. The PCR products sizes are converted into allele types using special software, which lets scientists determine how closely they are related.
CDC

PulseNet

- PulseNet USA is made up of 87 federal, regional, state, and local laboratories. There is at least one PulseNet laboratory in every state.
- In 2012, participating laboratories reported over 60,000 isolates to PulseNet. Over 4,000 of these isolates were from food, animal, or environmental sources.
- Each year in the United States, 1,200 clusters of disease are identified by state and local health agencies and 10—15 multistate or national outbreaks are identified.

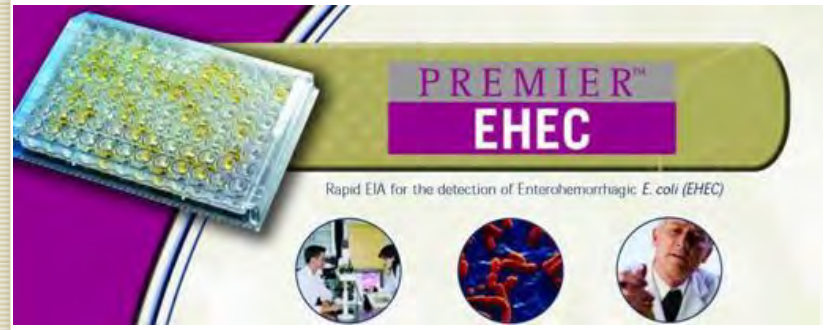
PulseNet

- PulseNet tracks subtypes of:
  - *E. coli* O157 and other Shiga toxin-producing- *E. coli*
  - *Campylobacter jejuni*
  - *Clostridium botulinum**
  - *Listeria monocytogenes*
  - *Salmonella*
  - *Shigella*
  - *Vibrio cholerae*
  - *Vibrio parahaemolyticus*

Available Testing Options

- ELISA's
- RCA's
- CIDT's
- Future?

ELISA



Meridian Bioscience, Inc.
Inspired Science. Trusted Solutions.

ProSpecT

- Common procedure and reagents for all tests
- Flexible specimen transport options
- Results within 100 minutes
- Visual or spectrophotometric reading

Thermo SCIENTIFIC

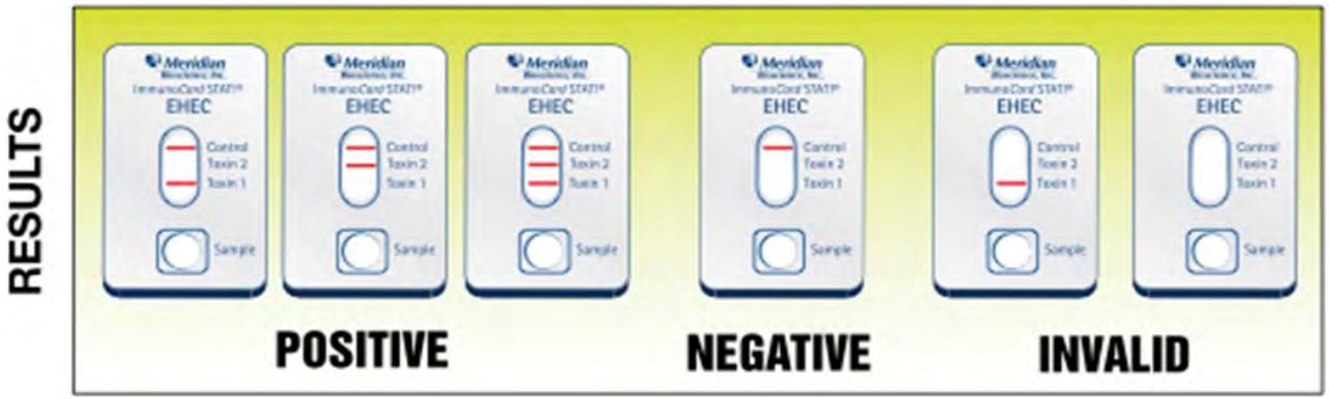
Rapid Cartridge Assays

DEFINITIVE ANSWERS, CONFIDENT RESULTS

- Fully comply with CDC guidelines
- Detects ALL Shiga toxin-producing *E. coli*, not just *E. coli* O157:H7
- Rapid identification to avoid contraindicated antibiotic therapy

Meridian Bioscience, Inc.
Inspired Science. Trusted Solutions.

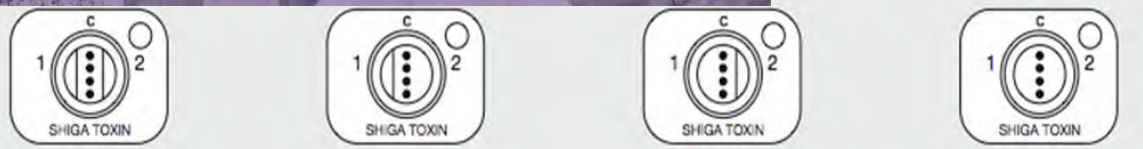
ImmunoCard STAT!



Rapid Cartridge Assays

Distributed by: Alere

Developed and Manufactured by: TECHLAB



Positive STX1 Positive STX2	Positive STX1 Negative STX2	Negative STX1 Positive STX2	Negative STX1 Negative STX2

Direct Fecal Testing Results

Culture-Independent Diagnostic Tests (CIDT)

- They have arrived!
- Two multiplex GI panel tests are now available for use (Luminex xTAG GPP and Prodesse ProGastro SSCS)
- Many others at various stages of development
- Will change the landscape of GI pathogen diagnostics and surveillance

Luminex xTAG GPP-ASR/RUO Kits

NEW *xTAG® Gastrointestinal Pathogen Panel (GPP) for* In Vitro *diagnostic use*

Multiplexed molecular testing for a reliable, actionable result in *under 5 hours*

- xTAG GPP is the only test that simultaneously detects and identifies the bacterial, viral, and parasitic pathogens responsible for over 95% of cases of infectious diarrhea[1]
- xTAG GPP provides:
  - More answers per sample than any other method
  - A reliable answer, in time to affect patient care
  - Better use of time and human resources

xTAG GPP – More results per test than any other method, in time to affect patient care

Method	Tests for	Turn-around time	Diagnostic yield
Stool culture	Single bacterial pathogen per test	2–3 days	Up to 6%[2]
Ova and parasite (O&P) exam	Parasitic pathogens	Several days – sample must be collected over 3 days	Up to 3%[3,4]
Rapid tests	Single pathogen per test	20–30 min	Varies
Real-time PCR	1–3 pathogens per test	Under 5 hours	Varies; depends on the pathogen target, individual performance and number of assays
ELISA	Single antigen/antibody per test	6–24 hours	Varies
xTAG GPP	Up to 15 bacterial, viral, and parasitic pathogens in a single test	Under 5 hours*	30%

*Including extraction steps

xTAG GPP – the only available gastrointestinal infection diagnostic that detects bacterial, viral and parasitic pathogens in a single test

Prodesse ProGastro SSCS GI Panel

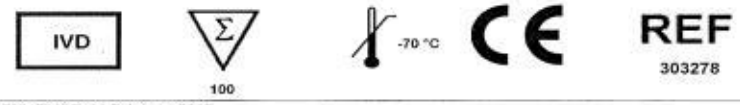
HOLOGIC | GEN-PROBE

Prodesse®

ProGastro™ SSCS

Instructions for Use

For the detection and differentiation of Salmonella, Shigella, Campylobacter (C. jejuni and C. coli only, undifferentiated) nucleic acids, and Shiga Toxin Producing E. coli (STEC) Shiga Toxin 1 (stx1) and Shiga Toxin 2 (stx2) genes.



503156 V.B 14 February 2013

bioMérieux (BioFire) GI Panel

FilmArray Gastrointestinal Panel

1 Test. 23 Targets. All in about an hour.

Bacteria

Aeromonas
Campylobacter
Clostridium difficile (Toxin A/B)
Plesiomonas shigelloides
Salmonella
Yersinia enterocolitica
Vibrio
Vibrio cholerae
Diarrheagenic E. coli/Shigella
Enteroaggregative *E. coli* (EAEC)
Enteropathogenic *E. coli* (EPEC)
Enterotoxigenic *E. coli* (ETEC) *lt/st*
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*
E. coli O157
Shigella/Enteroinvasive *E. coli* (EIEC)

Protozoa

Cryptosporidium
Cyclospora cayetanensis
Entamoeba histolytica
Giardia lamblia

Viruses

Adenovirus F 40/41
Astrovirus
Norovirus GI/GII
Rotavirus A
Sapovirus

The Future of "DNA Fingerprinting"

- Whole genomic sequencing
  - Requires pure cultures of bacteria
  - Use whole genome sequencing which by itself is a very powerful DNA fingerprinting method to help identifying and investigating outbreaks
  - Identify genetic targets for surveillance
  - Build reference database for metagenomics

The Future of "DNA Fingerprinting"

Key Factors	Next Generation Sequencing (NGS)	Current "Gold Standard" (PFGE)
Time to get data	1-2 days	Days
Time to analyze data	Hours to days	Hour or less
Cost per bacteria isolate (for reagents only)	~$150-300 (depends on platform)	~$10-15
Ability to differentiate and cluster isolates	To be determined but likely very good	Well-established for bacteria tracked by PulseNet, few exceptions
Potential for automation	Yes	No
Intended objective	Genetic serotyping, virulence, antimicrobial resistance profiling, and subtyping possibly based on SNPs or other variant regions	Only subtyping

The Future of "DNA Fingerprinting"

- Metagenomics
  - No pure culture of bacteria required
  - Sequence select genetic targets or all bacteria in a patient sample (stool, blood, etc.)
  - Identify bacteria making a patient sick and subtype at the same time

Studies

2011-2012 FoodCORE Study

- **FoodCORE**: **Food**borne diseases **C**enters for **O**utbreak **R**esponse **E**nhancement
- Public Health systems (Lab, Epi and Environ) granted funding to improve and model enhanced foodborne disease diagnostics, surveillance and prevention of further disease
- WI was one of 7 systems awarded funding in 2009; began laboratory activities in 2010
- Addressing STEC disease is one of the major foci for WI FoodCORE activities

2011-2012 FoodCORE Study

- Three clinical sites not performing routine Shiga toxin (Stx) screening of stool specimens were enlisted to participate
  - **Site 1** inoculated GN enrichment broth and sent to WSLH for microplate EIA Stx screening
  - **Site 2** performed microplate EIA screening for Stx in-house
  - **Site 3** performed Stx screening using a lateral flow rapid cartridge assay

2011-2012 FoodCORE Study

- Total of 8180 stools were screened for Stx from the three clinical system sites (Mar 1, 2011-Dec 31, 2012)
- There were 83 primary STEC isolates detected, of which 46 were O157 and 37 non-O157 STEC
- STEC recovered from the three clinical system sites represented 15% of the O157 STEC and 12% of the non-O157 STEC reported in WI over the study period (14% of total STEC WSLH rec'd)

STEC isolated during study:

- *E. coli* O5:NM
- *E. coli* O121:H undetermined
- *E. coli* O145:NM
- *E. coli* O26:NM and H undetermined
- *E. coli* O146:H21
- *E. coli* O103:H undetermined
- *E. coli* O157:H7 and O157:NM
- *E. coli* O45:H2 and H undetermined
- *E. coli* O111:NM
- *E. coli* O91:H14
- *E. coli* O undetermined: H undetermined*

*CDC unable to serotype

2011-2012 FoodCORE Study

- All of the non-O157 STEC and 6% of the O157 STEC (negative by culture at the clinical system site) would have been unreported had the three clinical system sites not been screening for Stx
- Of the 75 cases (out of 83) for which epidemiological data is available, there were 23 hospitalizations (18 O157, 5 non-O157), no deaths or HUS cases and the median age was 19 (age range from 0-89 years)

2011-2012 FoodCORE Study

- Positivity rates for Stx among all three clinical system site stool specimens averaged near 1% over the study period
- There were a total of 6 O157 STEC isolates (7.2%) during the study period that were included in national PulseNet clusters; no non-O157 STEC were included in national clusters
- Both the microplate EIA and RCA Stx diagnostic assays performed well; of all positive GN broths, WSLH isolated STEC from 92%

It Takes Teamwork…

Effective STEC disease diagnosis, surveillance and prevention takes a concerted effort by clinical health systems, public health laboratories, public health epidemiologists and env. health officials

How do We Partner to Prevent STEC and Other Diseases?

Gemplers.com

Extremehealthradio.com

Role of Clinical Health Systems

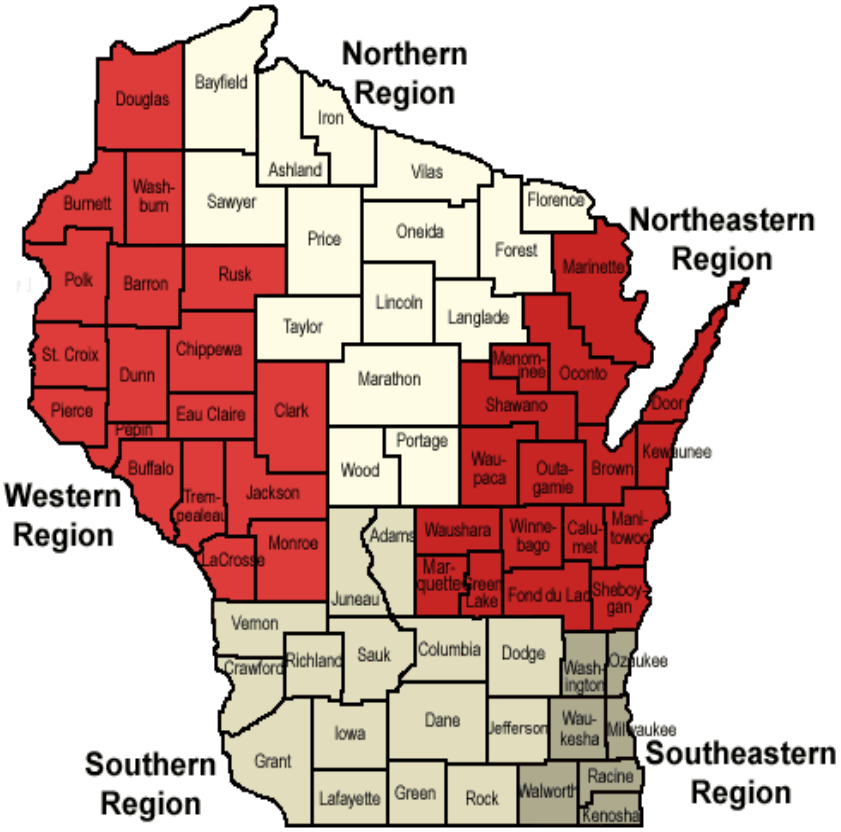
- Disease diagnosis and patient management is the primary focus; Clinical laboratories will generally be the first to see infectious disease cases
- Responsibility to notify local health officials, within the patient jurisdiction, of reportable disease cases as they detect them
- Prevention of spread of further communicable disease from index case(s)
- Responsible use of antibiotics; reduce development of antimicrobial resistance

Role of WSLH

- Laboratory service for the Wisconsin Division of Public Health (WDPH)
- Laboratory response to clusters of disease or pathogens of public health significance; emergency response
- Surveillance for public health pathogens
- Antimicrobial resistance monitoring
- Public health education and safety
- Research and specialized testing

Role of Local Health Officials

- Receive reportable disease information from clinical health systems or the public within their jurisdiction
- Investigate disease cases or clusters
- Disseminate disease information to WDPH



Role of WDPH

- Receive disease reports from local health, clinical health and environmental health officials
- SOS (Surveillance and Outbreak Support) Team will interview and enter epi data from cases in real time into a database; Epi's often have data in hand when they receive lab data from WSLH

Role of WDPH

- Receive lab data (PFGE subtyping, AST, Stx) from WSLH
- Work with other state and CDC epi's as needed to respond to regional or national clusters of illness
- Lead and coordinate cluster investigations
- Public Health education

Role of Environmental Health Sanitarians

- Work closely with Dept of Agriculture, Trade and Consumer Protection officials
- Conduct environmental health inspections and related activities for locations such as:
  - Restaurants
  - Hotels
  - Swimming pools and beaches

- Enforce public health laws and sanitary codes
- Investigate outbreaks, injuries, exposures, etc

2006 Multi-State Spinach E. coli O157: H7 Outbreak

O157:H7 Spinach Outbreak-2006

- Cluster of *E. coli* O157:H7 detected by PFGE subtyping in WI
- Reports to WDPH of multiple cases of HUS in the Milwaukee area
- Total of 204 cases of *E. coli* O157:H7 attributed to the spinach exposure; 49 of which were WI residents
- There were 104 patients hospitalized (51%), 31 cases of HUS (16%) and 3 deaths (1 WI)

O157:H7 Spinach Outbreak-2006

- Epi investigation traced source back to spinach field in CA
- Environmental sampling in the area led to the *E. coli* O157:H7 outbreak strain being detected from cattle on an adjacent farm, wild pig feces in the field and water in an irrigation channel in the field (confirmed by PFGE and MLVA subtyping)

O157:H7 Spinach Outbreak-2006

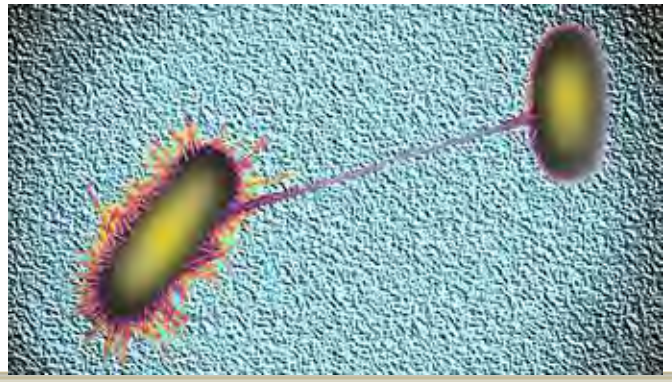
- Interestingly, one WI case was also determined to have a dual STEC infection; the outbreak O157:H7 and a non-O157 STEC
- NM state laboratory isolated the same non-O157 STEC from a bag of the implicated spinach
- How many other non-O157 STEC infections were there from the implicated spinach exposure?

O104:H4 STEC Outbreak

- May 2011 cluster of HUS cases detected in Germany
- No equivalent of PulseNet in Germany; Surveillance based on HUS case reporting
- Almost 4000 cases, 1000 HUS cases and 50 deaths attributed to the outbreak in the end
- Eventually linked to contaminated sprouts germinated from fenugreek seeds that originated in Egypt

O104:H4 STEC Outbreak

- O104:H4 novel strain; only seen in 2006 Japan and 2009 Rep. of Georgia human illness cases
- High interest in enhanced virulence ; genomic sequencing revealed the origin of this strain was likely an O157:H7 strain exchanging genetic material with an EAEC O104:H4 in the gut of a human



O104:H4 STEC Outbreak

Emerging Pathogens: Is E. Coli O104:H4 the Next Strain to Watch? | Food Safety News - Windows Internet Explorer

http://www.foodsafetynews.com/2013/09/e-coli-o104h4-the-next-strain-to-watch/

o104 emerging

File Edit View Favorites Tools Help

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Emerging Pathogens: Is E. Coli O104:H4 the Next Str...

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Emerging Pathogens: Is E. Coli O104:H4 the Next Strain to Watch?

BY JAMES ANDREWS | SEPTEMBER 9, 2013

In May 2011, a virtually unknown strain of E. coli, known as O104:H4, made worldwide headlines when an outbreak in Germany sickened approximately 4,000 people and killed 50, including one American. This event, linked to fresh sprouts, quickly became the deadliest foodborne illness outbreak in history.

In the days following the first reports of illness, microbiological research laboratories around the globe began pooling resources to coordinate the world's first "open source" analysis of a microbial genome. In turn, this spurred an unprecedented level of focused study by international collaborators that is still going on two years later.

Before the German outbreak, the only reported illness associated with O104:H4 was in South Korea and involved a solitary case of hemolytic uremic syndrome, or HUS, a potentially fatal kidney disease associated with severe E. coli infections.

Little to no research had focused on the rare strain leading up to its record-setting outbreak. But, as soon as it hit, O104:H4 rose in notoriety to a status reserved for E. coli O157:H7, the strain that has caused numerous deadly outbreaks in the past two decades and made E. coli a household name.

Thanks to recent technological advancements in DNA sequencing, researchers around the world were able to map the genome of O104:H4 within days – a feat that would have taken months if the outbreak had happened a decade earlier.

By working together to analyze the genome, researchers could more quickly determine why O104:H4 might be so particularly deadly.

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http://www.foodsafetynews.com/files/2013/08/E.-coli.jpg

Internet 100%

O45 STEC Associated with a WI Meat Processer- 2010-12

- Dec 2010, WSLH identifies matching/ closely matching PFGE patterns for four O45 STEC isolates (3 WI residents and 1 MI resident)
- Epi investigation leads to 3 more probable cases (1WI and 2 MI) and an interesting foodborne exposure scenario…

O45 STEC Associated with a WI Meat Processer- 2010-12

- Case patient food exposure histories lead to consumption of bear meat sticks as illness source
- Meat sticks were prepared with beef and bear meat
- Venison contamination found in beef sausage
- True source (beef, bear, venison)?

O45 STEC Associated with a WI Meat Processer- 2010-12

- WDPH Epi's thought this investigation was in the books but…
- Nov 2011, a new O45 STEC isolate with the same PFGE pattern is identified at WSLH
- Epi investigation finds that patient had venison processed at the same meat processor implicated in the 2010 outbreak

O45 STEC Associated with a WI Meat Processer- 2010-12

- Fast forward to May 2012: Dane county resident O45 STEC isolate determined to match the outbreak strain by PFGE
- Epi investigation by WDPH and local HD reveals (you guessed it) this patient also had venison processed at the same meat processor
- Health officials determined to find source of these infections

O45 STEC Associated with a WI Meat Processer- 2010-12

- In addition to environmental sampling, all employees were screened for Stx
- One employee Stx positive in June 2012
- Interestingly though, the culture yields an O146:H21 STEC, not O45 STEC as was suspected
- To date, no further O45 STEC cases linked to the meat processor…but hunting season begins again next Fall!

WI Tiger Meat Outbreaks- 2012 and 2013

WI Tiger Meat Outbreaks- 2012 and 2013

- It's a Holiday Tradition!
- Jan 2013, two cases of *E. coli* O157:H7 with matching PFGE patterns reported to WDPH; two more would be linked later
- Epi investigation links the cases to raw ground beef purchased at the same meat market
- 17 cases would be identified with raw beef exposure (14 consumed/ 3 cross contam.)

WI Tiger Meat Outbreaks- 2012 and 2013

- Dec 2013…It's still a Holiday tradition!
- 3 *E. coli* O157:H7 STEC isolates identified that have matching PFGE patterns
- Epi investigation determines 2/3 had consumed raw ground beef as Tiger Meat/ cannibal sandwiches
- Definitive source of beef not identified after a thorough investigation by PH officials

Culture Independent Diagnostic Tests- Impact on Patient Mgmt

- In many cases, will detect pathogens more quickly than traditional detection methods
- Multiplex assays will pick up multiple pathogens in a single specimen...clinical significance of one, both, all?
- Loss of isolate for antimicrobial susceptibility testing (if needed)

Culture Independent Diagnostic Tests- Impact on Public Health

- Potential loss of isolates if clinical health system discontinues enteric bacterial culture (current basis for surveillance)
  - Loss of isolate for serotyping and subtyping
  - Loss of isolate for antimicrobial resistance testing
- Greater resources necessary to perform enteric culture on specimens received from clinical health systems

Specimen Submissions

- As with other enteric specimens, STEC specimens may be submitted to WSLH via overnight courier as part of the **W**isconsin **E**nteric **P**athogen **S**urveillance (WEPS) Program
- Contact Dunham Express courier service: 800-236-7127 (account 7271)
- For technical questions, call WSLH: 800-862-1013

CDD Requisition Form A

Bacteriology:		
MP00470		Bacterial ID, Non-Enteric Public Health Panel (WEIP); Suspect Organism: _____
MP00628		Bacterial Typing (PFGE) Organism _____
MP00623		Bordetella Culture
MP00311		Bordetella PCR
		Botulism Testing (Call 800-862-1013)
SC00111		Chlamydia/GC NAAT
SC00118		Chlamydia trachomatis NAAT
SC00112		Neisseria gonorrhoeae NAAT
VR01502		Chlamydia trachomatis Culture
MP00681		CRE (KPC/ NDM-1) PCR Organism _____ Attach Susceptibility Results
MP00380		Diphtheria Culture
MP00460		Enteric Pathogen Isolate Identification (WEPS) Suspect Organism _____
MP00610		Haemophilus ducreyi Culture
MP00420		Legionella Clinical Culture
MP00421		Legionella PCR
MP00549		Shiga Toxin EIA
MP00536		Shiga Toxin PCR Screen
MP00684		S. aureus for VISA/VRSA Confirmation
MP00660		Stool Culture, Routine Suspect Organism _____

Bacteriology:		
MP00593		Toxic Shock Syndrome Toxins
BT Agent (Potential), please call 800-862-1013		
WSLH assigned		*Suspect Agent (please circle)* • Burkholderia mallei/pseudomallei • Bacillus anthracis • Francisella • Brucella • Yersinia pestis
Invasive Bacteriology Surveillance (IBLS):		
MP00666		Group A Beta Streptococcus
MP00666		Group B Beta Streptococcus
MP00651		Haemophilus influenzae
MP00628		Listeria monocytogenes
MP00561		Neisseria meningitidis
MP00465		Streptococcus pneumoniae
Mycobacteriology:		
MM00250		Mycobacteria (AFB) Smear and Culture
MM00253		Mycobacteria Isolate Identification
MM00202		Mycobacterium avium Complex (MAC) Susceptibility
MM00204		Mycobacterium tuberculosis Susceptibility- 1st Line Drugs
MM00207		Mycobacteria Rapid Grower Susceptibility
MM00256		Mycobacterium tuberculosis PCR Decontaminated? Yes No Smear Result _____

Parasitology:		
MP00800		Arthropod Identification
MP00802		Cryptosporidium/Giardia DFA
MP00840		Ova & Parasites, Intestinal
MP00880		Parasites Blood Smear Suspect Parasite _____
MP00881		Parasites Tissue Smear Suspect Parasite _____
MP00860		Worm Identification
Virology:		
VR01703		Enterovirus PCR (CSF)
VR01704		Herpes Simplex Virus PCR (Genital/Dermal)
VR01705		Herpes Simplex Virus PCR (CSF)
VR01713		Measles Virus PCR
VR01714		Mumps Virus PCR
VR01717		Norovirus PCR
VR01725		Rubella Virus PCR
VR01727		Varicella Zoster Virus PCR
VR01725		Rubella Virus PCR
VR01727		Varicella Zoster Virus PCR
Other Tests (Specify):		

WHITE–RETURN TO WSLH
YELLOW–KEEP FOR YOUR RECORDS

Summary

- STEC disease is a significant cause of morbidity and mortality in WI and nationally; there are significant costs, both human and monetary
- here are multiple diagnostic testing options available for Stx detection with many more in development
- WI clinical health systems have been testing for Stx for many years; currently there is effective statewide Stx diagnostic testing which is critical for STEC diagnosis, surveillance and prevention

Summary

- CIDT's have proven beneficial to STEC and other disease diagnoses; however they have the potential for adverse impact on current isolate-based public health surveillance systems
- Effective STEC disease detection, surveillance and prevention requires a strong partnership among clinical and public health agencies and officials; we believe such a partnership exists today in WI

Acknowledgements

- Rachel Klos, Traci DeSalvo and Justin Kohl- WDPH state foodborne disease epidemiologists
- Three FoodCORE STEC enhancement initiative sites:
  - Marshfield Clinic
  - St. Mary's Hospital- Madison
  - Fort Healthcare
- Colleagues in the WSLH Bacteriology and PFGE laboratories
- Clinical health systems and laboratorians in WI

Contact Information

Mike Rauch

Advanced Microbiologist

michael.rauch@slh.wisc.edu

Tim Monson

Microbiologist Supervisor

timothy.monson@slh.wisc.edu

WSLH CDD Customer Service (800-862-1013)

Questions?