## Shiga Toxin-producing *E. coli* (STEC), O157 and Non-O157: Efforts in Wisconsin to Improve Detection

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**WSLH Audio Conference Program**
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## Objectives

- Describe the epidemiology, pathogensis and clinical manifestations of STEC disease
- Describe various testing protocols for the detection of STEC infection
- Describe testing available at WSLH for the detection of STEC infection
- Describe a state-wide collaborative testing program for STEC detection and surveillance

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**All E. coli are not created equal**

- Several *E.coli* groups can cause outbreaks of severe diarrhea:

| | |
|---|---|
| STEC, EHEC | enterohemorrhagic (ie: O157:H7) |
| ETEC | enterotoxigenic |
| EPEC | enteropathogenic |
| EAEC | enteroadherant |
| EaggEC | enteroaggregative |
| EIEC | enteroinvasive |
| DAEC | diffuse adherant |

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## History

- 1885----E. coli first described
- 1982----E. coli O157:H7 first recognized as a human pathogen
- 1985----Karmali linked toxin-producing E.coli to hemolytic uremic syndrome (HUS)
- 1993----Outbreak in four western states affecting 500+ persons, 56 HUS, 4 deaths
  - Led to recommendations for screening

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## STEC

- A shiga toxin-producing E. coli (STEC)
  - AKA verotoxin, verocytotoxin, shiga-like toxin
  - Stx1 and Stx2
  - Similar to shiga toxin produced by *S. dysenteriae*
- Possess other virulence factors
  - Attaching and effacing gene (eae)
  - Enterohemolysin gene (E-hly)
- Other E. coli serotypes can produce shiga toxin
  - O26:H11 O145:NM O45:H2
  - O111:H8 O118:H2 O104:H21
  - O113:H21O121:H19
  - >100 serotypes

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## E. coli O157:H7

- O157:H7 and O157:NM
  - 73,000 cases of illness each year in U.S.
  - 60 deaths
  - 80% of cases of hemolytic uremic syndrome (HUS)
- Prevalence in Wisconsin----CHOW
  - Kehl et. al (JCM 35:2051-54, 1997)
    - E. coli O157:H7----------1.2%
    - Non-O157 STEC----------0.3%
    - Salmonella----------2.8%
    - Shigella----------3.1%
    - Campylobacter----------0.9%

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## Cases of enteric pathogens reported in Wisconsin 2002

- Campylobacter----------1159
- Salmonella---------- 876
- E. coli O157:H7---------- 293
- Shigella---------- 178
- non-O157:H7 STEC-------- 5

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## Surveillance

- 1993--CSTE recommended that infections be made reportable by all states
- 2000--Made a reportable disease in Wisconsin
- Nationally reportable disease
- Part of the CDC PulseNet Program
  - Established 1996

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## Clinical Aspects

- Incubation period
  - Typically 2-4 days, range 1-7
  - HUS in 5-10% of infected
    - presents 2-10 days following the initial infection
- Initial symptoms
  - Diarrhea---mild, watery, bloody
    - Fecal leukocytes in >70%
  - Abdominal cramps
  - Vomiting in about 50% of patients

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## Hemolytic Uremic Syndrome

- More frequent in young (<5 years) and the elderly (>75 years)
  - Unclear why, but may be related to lack of immunity
- Triad of characteristics
  - renal failure
  - thrombocytopenia
  - hemolytic anemia

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## Outcome of HUS

- 5-10% mortality
  - Multi-organ failure---CNS, pancreas, heart, and others
- Long-term complication rate of 30-50%
  - Chronic renal failure
  - proteinuria
  - Hypertension
- Stroke----3-4%

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## Other complications

- Thrombotic thrombocytopenic purpura (TTP) much like HUS except:
  - occurs in a more adult population
  - associated with neurological complications
- Thrombotic strokes
- Blindness
- Coma
- Cardiac failure
- Gut perforation, gangrene

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## Pathogenesis

- Colonize portions of the lower intestine
  - Capacity to adhere, attach, and efface epithelial cells important
- Production of Shiga-toxin (STEC)
  - Cross intestinal epithelium, reaching sub-epithelial tissues
- Endothelial cells lining small blood vessels are final targets for the toxins
  - kidney, brain, and other organs

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## Pathogenesis (2)

- Formation of aggregates of platelets within blood vessels with generation of thrombotic microangiopathy

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## Transmission

- Cattle primary reservoir
  - Prevalence can reach 63-100% in feedlots
  - Part of normal intestinal flora
  - No clinical illness
- Contamination of meat during slaughter
  - Spread through grinding and mixing
- Transmission to humans
  - Consumption of undercooked beef or beef products
  - Cross contamination of other foods

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## Transmission (2)

- Cases or outbreaks associated with
  - Beef, salami, sausage, other meat products
  - unpasteurized milk
  - unpasteurized apple juice/cider
  - lettuce
  - alfalfa and radish sprouts
  - water---unchlorinated drinking and recreational
- Person-to-person transmission
  - Shedding—most <1 week; some >3 weeks

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## Outbreaks

- 52% Cattle-related food
  - 45% ground beef
- 14% Fruits and vegetables
- 12% Water
- 2% Milk
- 16% Direct transmission
  - person-to-person
  - cattle-to-person

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**Important Outbreaks of non-O157:H7 STEC**

- Australia, 1995 (see Paton *et al*., JCM 34:1622-7)
  - >160 cases
  - 22 HUS, 4 TTP
  - O111:H-, stx1+, 2+, eae+ (PCR)
  - Mettwurst (dry, fermented sausage) was infection source
- Texas, 1999 (see MMWR 49:321-4)
  - 58 cases,
  - 2 HUS
  - O111:H8, stx1+, 2+ (PCR and EIA)
  - Ice in drinking water implicated as source
- Outbreaks of sorbitol-fermenting O157:H- in Europe (see Ammon *et al*., JID 179: 1274-7)

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## Wisconsin Outbreaks
## E. coli O157:H7

- Ozaukee County Fair, Cedarburg, August 2001
  - E. coli O157:H7
  - Associated with contact with cattle
  - 25 lab confirmed cases
  - 34 probable
  - No HUS or fatalities
  - Led to state guidelines for exhibit of animals

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## Wisconsin Outbreaks (2)
## E. coli O157:H7

- UW-Madison Pancake Breakfast, October 2001
  - Held in the stock pavilion on campus
  - 34 ill, 16 confirmed by culture
  - 1 child with HUS
  - No food implicated
  - Pavilion houses animals
    - 3 days previous, cows housed in a pen in the pavilion where the foodline was located
    - Outbreak strain isolated from environment and one of the cows

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## Wisconsin Outbreaks (3)
## E. coli O157:H7

- 2 Multi-county, multi-state outbreaks, Summer 2001
  - 9 states involved in one outbreak, 13 in other
  - 2 different strains of E. coli O157:H7
  - 54 lab confirmed WI cases
  - 44 cases from other states
  - 1 HUS, no fatalities
  - Associated with consumption of ground beef
    - One outbreak strain isolated from ground beef
  - Led to meat recall

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## Proportion of STEC isolates that were O157 vs non-O157, diarrheal stools

| State | STEC | O157 | non-O157 |
|---|---|---|---|
| MN | 134 | 74 (55%) | 60 (45%) |
| CT | 32 | 19 (59%) | 13 (41%) |
| NM | 35 | 14 (40%) | 21 (60%) |
| Neb | 14 | 7 (50%) | 7 (50%) |
| Total | 215 | 114 (53%) | 101 (47%) |

- MN – Besser et al., unpublished
- CT - Florentino et al., ASM 2001 (Abstract C166)
- NM – Nims et al. ASM 2001 (Abstract C165)
- Neb – Fey et al. EID 6:530-533, 2000

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## CDC Recommendations (MMWR, 4/21/00)

- Clinicians should inform health departments about clusters of bloody diarrhea and HUS
- Clinical Labs should screen bloody stools for STEC, and attempt to isolate STEC from positive stools
- Positive isolates should be referred to public health laboratories for serotyping and further characterization.
- States should consider adding STEC infection to their notifiable disease lists.

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## Laboratory Diagnosis of STEC: Culture for *E. coli* O157:H7

- Serotype O157-Specific Culture Methods
  - Selective and differential media
  - **S**orbitol **Mac**Conkey agar plates (SMAC)
  - **C**efixime-**T**ellurite **S**orbitol **Mac**Conkey Agar (CT-SMAC)
  - Chromagars:
    - Rainbow Agar O157
    - ChromAgar O157

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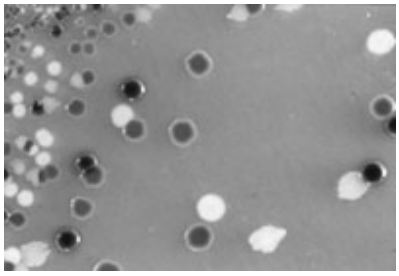
## Chromagar O157



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## Rainbow Agar

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## Laboratory Diagnosis of STEC: Culture for *E. coli* O157:H7

- *E. coli* O157:H7 either fail to ferment sorbitol or exhibit delayed sorbitol fermentation (rare exceptions occur)
- Non-O157 bacteria that grow on SMAC may also be sorbitol negative
- Must confirm using specific agglutination assays for O157 and H7 antigens

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## Laboratory Diagnosis of STEC: Culture for *E. coli* O157:H7

- Biochemical confirmation of *E. coli* O157:H7
  - **M**ethyl**u**mbelliferyl-β-D-**g**lucuronide (MUG) Test to test for the presence of β-glucuronidase enzyme (O157:H7 are typically MUG negative)
  - Common biochemical profile for O157:H7 strains

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## Laboratory Diagnosis of STEC: Disadvantages of Conventional Testing for *E. coli* O157:H7

- Sorbitol-fermenting E. coli O157:H7 strains
- Low numbers of O157:H7 may not be isolated
- Sensitivity of culturing for O157:H7 strains, using SMAC plate only, is estimated to be only 50-60%
- Non-O157:H7 STEC strains will not be detected

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## Laboratory Diagnosis of STEC: Enhanced Culture Method

- **Im**munomagnetic **S**eparation (IMS)
  - Metallic beads coated with anti-O (LPS) antibody (anti-O157, anti-O26, anti-O111,etc)
  - Mix beads with stool in enrichment broth
  - Beads specifically bind bacterial LPS
  - Immobilize beads with a magnetic field
  - Wash then plant beads on plate media
  - May increase sensitivity of culture 100-fold

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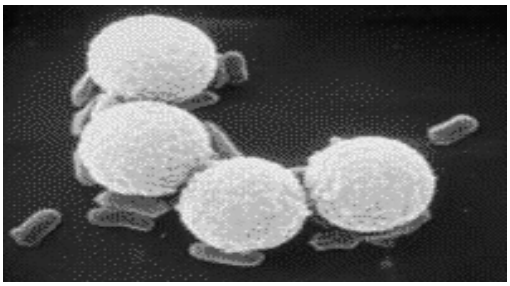
## Immunomagnetic Separation -Scanning electron-micrograph



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## Laboratory Diagnosis of STEC: Non-culture Methods

- Toxin Assays
  - Vero cells commonly used
- EIA
  - Meridian Premier EHEC
  - Alexon Trend ProSpecT Shiga Toxin E. coli (STEC)
- RPLA
  - Oxoid VTEC-RPLA

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## ImmunoCard STAT! E. coli O157:H7


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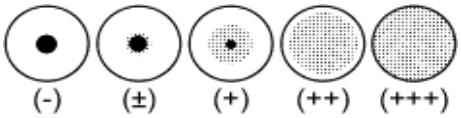
## Reversed Passive Latex Agglutination Assays

- Latex particles sensitized with anti-serum reactive with Shiga toxins (verocytotoxins) 1 and 2
- Agglutination of latex particles and toxin results in a lattice formation at the bottom of the V-shaped microtiter plate well

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## Laboratory Diagnosis of STEC: Non-serotype specific testing

- Non-serotype specific assays involve:
  (1) Detection of Shiga toxins (Toxin assays)
  (2) Detection of specific virulence genes associated with STEC disease (Genetic assays)
- Non-serotype specific assays will detect non-O157:H7 STEC strains as well as O157:H7 strains

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## Laboratory Diagnosis of STEC: Toxin Assays

- Cytotoxicity in cell culture
  - Vero cells commonly used
- Immunoassays
  - Commercially available--- Alexon-Trend, Meridian Biosciences, Oxoid
  - Test stools directly--- (70-80% sensitive)
  - Incubate in enrichment broth (GN or MAC) --- (approach 100% sensitivity)

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## Isolation and Confirmation of STEC: WSLH Testing Algorithm

- Stool specimen submitted
  (1) Plate to MAC, SMAC and CT-SMAC to rule out *E. coli* O157:H7
  (2) GN enrichment broth set up for Shiga toxin EIA testing
  (3) Shiga toxin EIA assay performed if culture negative for *E. coli* O157:H7
  (4) Enrichment broths positive for Shiga toxin are plated for PCR testing and isolation of STEC

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## Isolation and Confirmation of STEC: WSLH Testing Algorithm

- GN Enrichment broth (Shiga toxin positive) or isolate submitted
  (1) Plated to MAC, SMAC and CT-SMAC to rule out *E. coli* O157:H7
  (2) Sweep of growth from MAC or SMAC plate tested by PCR for the presence of Shiga toxin genes (*stx*1 and *stx*2)
  (3) If positive by PCR, individual colonies tested to find STEC

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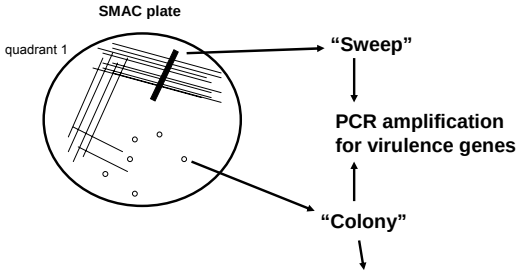
**STEC Testing Algorithm:**
**Isolation and Identification of STEC isolates**




- Biochemical testing and serotyping performed
- Molecular Subtyping (PFGE) and submission to CDC

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# E. coli O157 and STEC Virulence Genes

- *stx*1 Shiga toxin 1 (SLT-1/ VT 1)
- *stx*2 Shiga toxin 2 (SLT-2/ VT 2)
- *eaeA* *E. coli* attaching and effacing gene (Intimin)
- *uidA* O157 allele of beta glucuronidase
- E-*hly* Enterohemolysin (found on plasmid present in *E. coli* O157:H7 and other EHEC)

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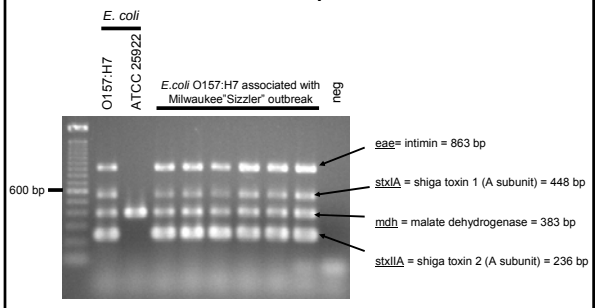
**"STEC Multiplex PCR"**

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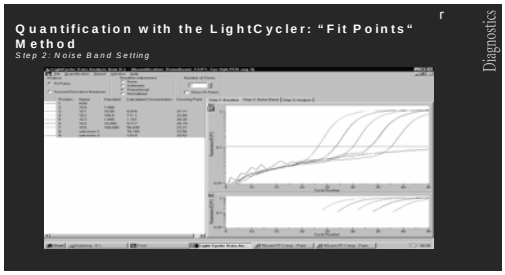
## Real-Time PCR Assay

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## Laboratory Diagnosis of STEC: Serotyping

- Slide Agglutination at WSLH
  - EPEC/VTEC Pool USA
    O45 and O121
  - EPEC/VTEC Pool 1
    O26, O103, O111, O145 and O157
- STEC sent to CDC for H antigen serotyping

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## STEC Serotypes isolated in WI

- O157:H7 (many)
- O28ac:H25
- O45:H(pending)
- O45:H2
- O103:H2 (4)
- O111:NM
- O157:NM (many)
- O145:NM (2)
- O?:H28
- O?:NM
- unnamed isolate
- O and H pending

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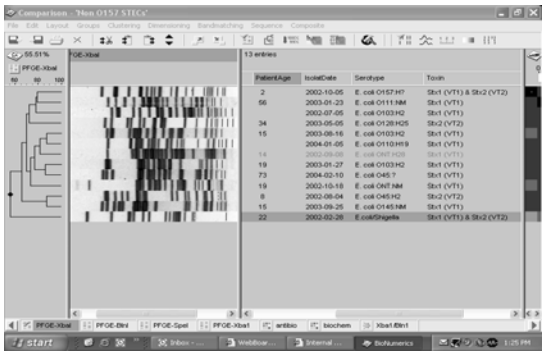
## PFGE of STEC Isolates



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## Wisconsin SHIELD Program

- **Shi**ga Toxin **E**pidemiology and **L**aboratory **D**etection Program
- Surveillance program to determine the prevalence of STEC disease in WI and identify/serotype the agents involved
- Participating laboratories are sending stool specimens, bacterial isolates and enrichment broths from patients that meet the program criteria

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## Wisconsin SHIELD Program: Submission Criteria

- Specimens from patients with a history of diarrheal illness that also:
  (1) Are suspected or confirmed HUS cases
  (2) Are suspected or confirmed of containing blood
  (3) Are from pediatric patients
  (4) Are known or suspected contacts of confirmed STEC cases or outbreaks

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## WSLH WEPS Program




## WEPS Program- STEC

- **W**isconsin **E**nteric **P**athogens **S**urveillance Program- requests submission of STEC specimens and other enteric pathogens to WSLH for PFGE analysis and antimicrobial resistance monitoring
- Shiga toxin EIA positive enrichment broths or resulting STEC isolate
- Confirmed or suspect STEC Isolates requested
  - *E. coli* O157:H7
  - Non-O157 STECS



## Submission of Specimens

- Dunham Express courier service is available for delivery of SHIELD or WEPS Program specimens to WSLH
  - -Account number 7271
  - -Call 1-800-236-7127

## References


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