Non-O157 Shiga toxin-producing *Escherichia coli*:

Isolation and detection challenges

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Outline

- Nomenclature (STEC, EHEC, VTEC) and abbreviations
- Isolation and detection challenges
  - Diagnostic methodology
  - Detection of outbreaks
  - Guidelines for laboratories and physicians
- Summary

Nomenclature

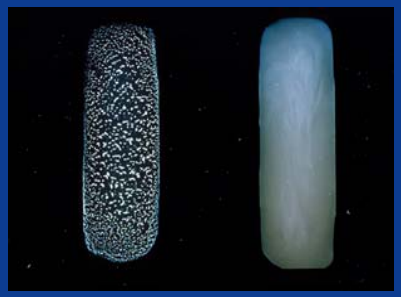
- Verocytotoxin-producing *E. coli* (**VTEC**)
  - Konawalchuck, et al, 1977
- Enterohemorrhagic *E.coli* (**EHEC**)
- Shiga toxin-producing *E. coli* (**STEC**)
  - O'Brien, et al, 1983
- Shiga toxin-producing E. coli (**STEC**) nomenclature used in this presentation

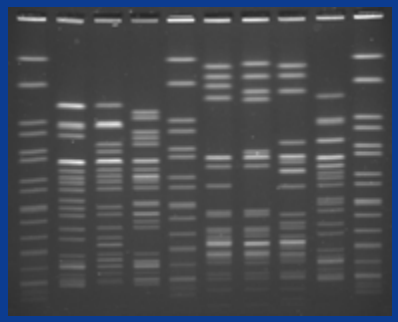
Abbreviations

- **O157 STEC**
  - Shiga toxin-producing *E. coli* O157:H7
- **Non-O157 STEC**
  - All other serotypes of Shiga toxin-producing *E. coli* (more than 100)
- **Stx**
  - Shiga toxin
- **Stx-EIA**
  - Shiga toxin immunoassay (test which detects the presence of Shiga toxin)
  - not all are enzyme immunoassays

Diagnostic methodology challenges

Diagnosis of O157 STEC





Stool Specimen

Colorless colony on SMAC agar

Agglutination in O157 antiserum

Pulsed field gel electrophoresis

PFGE Patterns to PulseNet

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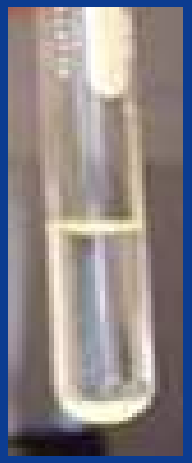
Diagnosis of non-O157 STEC

Stool Specimen



GN broth

Stx EIA

Note: no SMAC plate, no colony, no PFGE, no PulseNet

Diagnostic methodology challenges

- Non-O157 STEC
  - No useful isolation medium is available
  - Look like "normal" *E. coli*
    - media used for O157 STEC not useful
  - Stxs EIAs the only practical method for clinical diagnosis

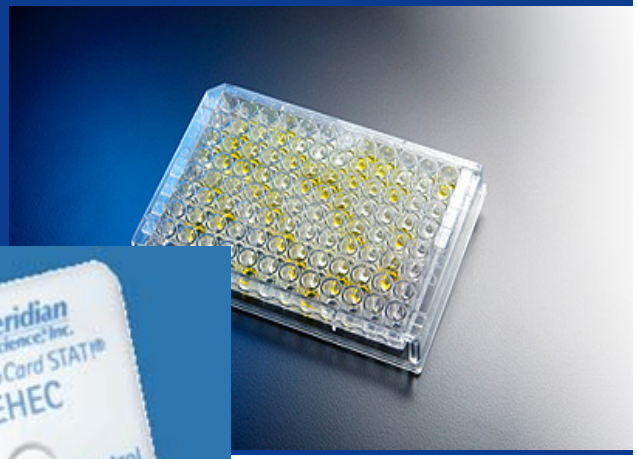
Sorbitol MacConkey agar (SMAC)

STEC Diagnosis: Disadvantages of Stx EIAs

- Stx EIAs cannot differentiate
  - between *E. coli* O157:H7 and other STEC serotypes
  - between Stx1 and Stx2 (more serious symptoms)
- False positive reactions are not uncommon
  - Inadequate plate washing
  - Visual reading not accurate
  - If inappropriate specimens are tested
  - Cross reactions with *Pseudomonas*, norovirus?

Commercial Stx EIAs

- Premier EHEC
- ProSpecT Shiga Toxin
- Duopath Verotoxin GLISA
- ImmunoCard STAT! EHEC
- BioStar OIA SHIGATOX



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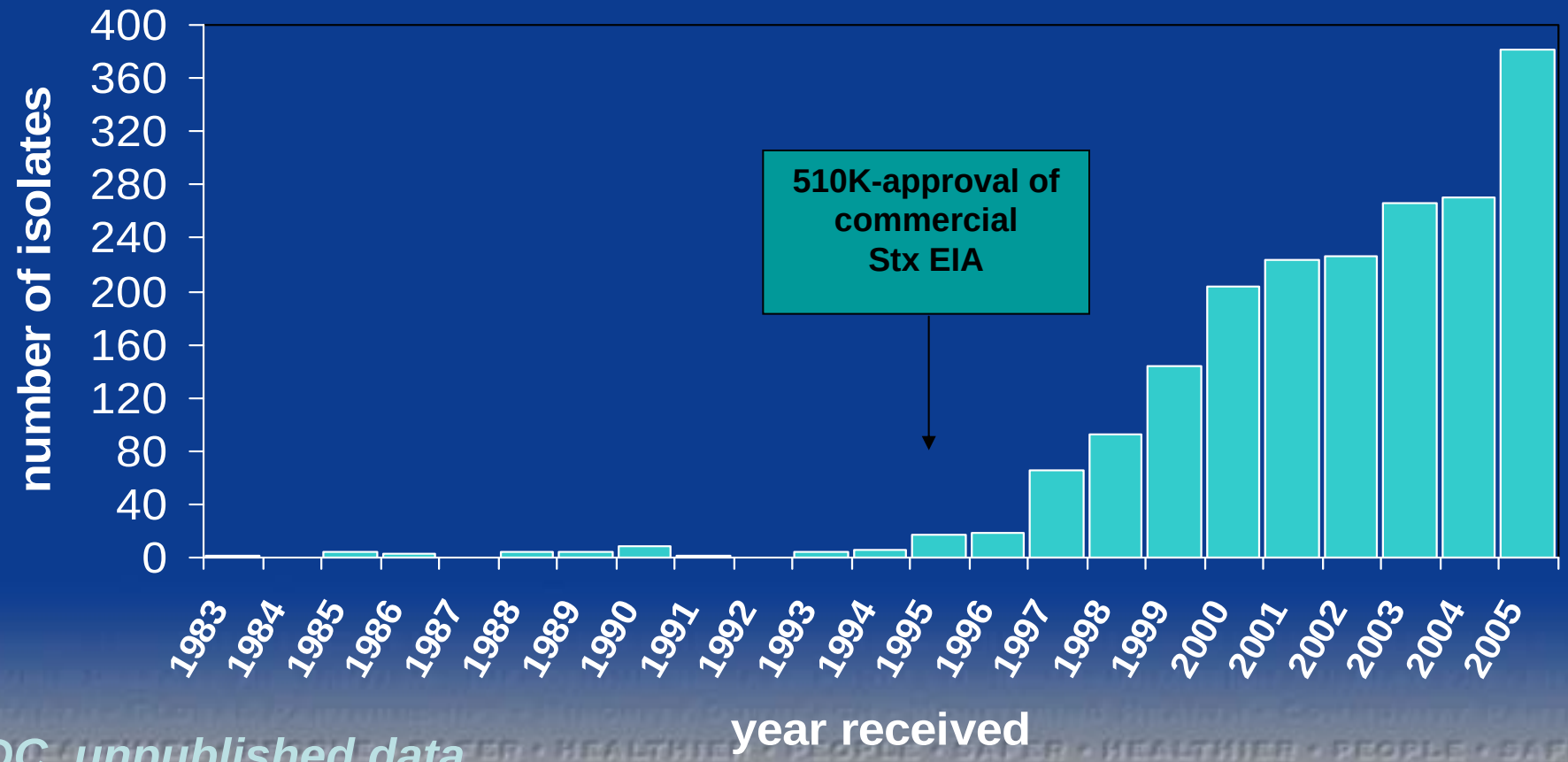
1,945 Non-O157 STEC Serotyped by CDC, 1983-2005



CDC, unpublished data

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Challenges for outbreak detection (how do you isolate non-O157 STEC?)

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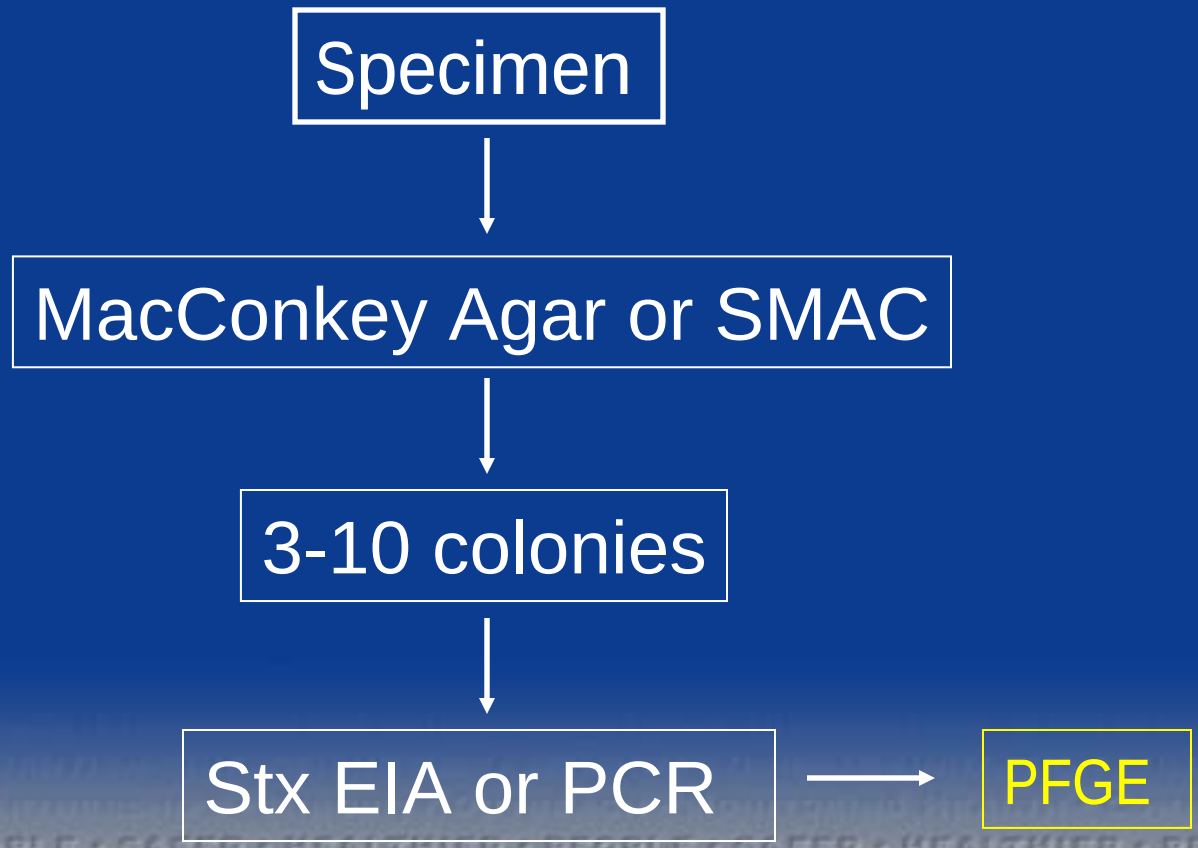
Isolation of non-O157 STEC



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Challenges for outbreak detection

- Most clinical laboratories don't attempt to isolate non-O157 STEC
  - Clinical labs send Shiga toxin positive broths to public health laboratories
- Public health laboratories
  - Most isolate non-O157 STEC from broths sent by clinical labs

Problems for Public Health Labs

- Testing broths for non-O157 STEC is expensive
  - Multiple isolates must be tested by EIA or PCR
  - Laboratory personnel and reagents are expensive
- Public health labs have other priorities
  - *Influenza*
  - *HIV and STDs*
  - *Tuberculosis*
  - *Bioterrorism*

Outbreaks of non-O157 STEC infections, United States*

Year	Serogroup	Exposure / Vehicle
1990	O111	Unknown
1994	O104	Milk
1999	O121	Lake water
1999	O111	Salad bar
2000	O103	Punch
2001	O111	Day care
2001	O26	Lake water
2004	O111	Apple cider
2005	O45	Food handler
2005	O26	Day care
2006	O45	Day care
2006	O121	Day care
2006	O121	Salad

Commercial Shiga toxin assay ←

**CDC, unpublished data*

More problems for public health labs

- When the public health lab gets different results from the clinical lab
  - How to interpret and report?
    - *A report that the broth is negative is also a problem for the clinical lab and the clinician*
  - Should a child be excluded from daycare?
  - Should a foodhandler be excluded from work?

A critical need: guidelines for laboratories and physicians

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Guidelines for laboratories and physicians

- Guidelines needed for diagnosis and detection of non O157 STEC
  - Physicians
  - Clinical diagnostic laboratories
  - Public health laboratories

Physicians need to know

- Must act *quickly* - only 3 to 4 days to prevent HUS
- Order appropriate diagnostic test and understand its utility and limitations

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Clinical labs need to know

- Clinical laboratories need specific guidelines for diagnostic testing
  - What specimens to test
  - What test methods to use
  - How to interpret and report results to physicians
  - *E. coli* O157:H7 isolates and Shiga toxin-positive broths should be sent to a public health laboratory

CDC Recommendations for Laboratory Diagnosis of STEC

September 29, 2006

MMWR™
Morbidity and Mortality Weekly Report

BOX. Recommendations for laboratory identification of Shiga toxin-producing *Escherichia coli* (STEC)

- Health-care providers should notify clinical diagnostic laboratories when STEC O157 infection is suspected (e.g., because of bloody diarrhea or hemolytic uremic syndrome) so that appropriate testing methods can be applied.
- Clinical diagnostic laboratories should strongly consider including STEC O157 in their routine bacterial enteric panel (with *Salmonella, Shigella*, and *Campylobacter*).
- The best way to identify all STEC infections is to screen all stool samples submitted for routine enteric bacterial testing for Shiga toxins (Stxs) using enzyme immunoassay (EIA) or polymerase chain reaction. Ideally, the clinical diagnostic laboratory should culture simultaneously for STEC O157 (e.g., on sorbitol MacConkey agar). Simultaneous culture facilitates rapid diagnosis and treatment of patients with STEC O157 infection and rapid subtyping by public health laboratories; such rapid action is most important when the index of clinical suspicion for STEC O157 is high.
- Clinical diagnostic laboratories that use an Stx EIA but do not perform simultaneous culture for STEC O157 should culture all Stx-positive broths for STEC O157 as soon as possible and rapidly forward these isolates to a state or local public health laboratory for confirmation and subtyping.
- When an Stx-positive broth does not yield STEC O157, the broth culture should be quickly forwarded to the state or local public health laboratory for identification of non-O157 STEC.
- State and local public health laboratories should confirm the presence of Stx in broths sent from clinical laboratories and should attempt to obtain an STEC isolate. All non-O157 STEC isolates should be sent by public health laboratories to CDC for confirmation and further characterization.

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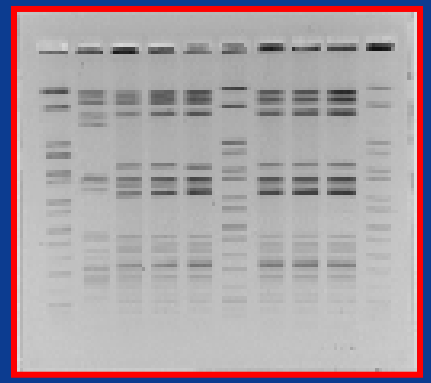
What else do clinical labs need to know?

- SMAC is not enough (only detects O157:H7)
- Commercial assays can produce false positives and false negatives
- Importance of promptly communicating positive results to the physician
- Participate in proficiency testing program (API, CAP)
- How can the laboratory be reimbursed for testing for non-O157 STEC?

What Public Health Labs Need to Know

- Timely culture of non-O157 STEC important for
  - outbreak detection
  - feedback to the submitting lab
  - feedback to physicians treating patients
- Allocate personnel and train them to isolate STEC from broths and stool
- Send non-O157 isolates to CDC for serotyping and confirmation



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What else do public health labs need to know?

- Large diagnostic labs are confused and frustrated about what type of Stx-positive specimens to public health labs
  - Broth?
  - Fecal specimen?
  - Isolate?
- Public health labs have different specimen submission rules

Guidelines for STEC specimen submission need to be developed

- Develop consensus guidelines for submission of broths and specimens for STEC testing
  - The Association for Public Health Laboratories (APHL)
  - Public health labs
  - Clinical diagnostic labs

Guidelines for STEC Diagnosis CDC Goals

- Develop consensus guidelines for isolation and identification of STEC with partners
  - APHL, ASM, Public Health Labs, Clinical Labs, Clinicians
- Develop interpretation guidelines for Stx EIA results

Summary

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Summary

- The challenges are daunting
  - No selective medium for non-O157 STEC
  - Lack of personnel and resources
  - Lack of clear guidelines for testing, interpretation of results, and reporting
  - Need for training of laboratory personnel
  - Need for standard state submission laws accessible to clinical lab personnel
  - Need to educate physicians about test availability, utility, and limitations
  - Etc.

Summary

- But there is remarkable cooperation among
  -commercial diagnostic laboratories
  -public health laboratories
  -APHL
  -clinicians
  -CDC
  to address these challenges!

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Thank you for your attention

The findings and conclusions in this presentation are those of the author and do not necessarily represent the views of the Centers for Disease Control and Prevention

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