Institutions Represented

- Government
- Non-governmental organizations
- International partners
- Medical device industry
- Commercial laboratories
- Healthcare institutions
- Professional societies
- Academic institutions
Forum Goals

- Raise awareness
- Define the issues
- Stimulate applied research
- Start building consensus
- Initiate post-meeting actions
Bacterial Culture
Rapid ("Culture-Independent"; "Non-Culture") Tests

(Pictures of commercial products removed)
Crisis

Danger + Opportunity
Danger: Threats to infectious disease surveillance programs

Opportunity: Potential solutions; benefits of action
<table>
<thead>
<tr>
<th></th>
<th>Culture</th>
<th>Rapid/culture-independent tests</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Speed</strong></td>
<td>Slow</td>
<td>Fast</td>
</tr>
<tr>
<td><strong>Infrastructure needed</strong></td>
<td>Significant</td>
<td>Minimal</td>
</tr>
<tr>
<td><strong>Expertise required</strong></td>
<td>Significant</td>
<td>Minimal</td>
</tr>
<tr>
<td><strong>Labor cost</strong></td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Cost of materials</strong></td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>
# Rapid / Culture-Independent Tests versus Culture

<table>
<thead>
<tr>
<th></th>
<th>Culture or standard tests (e.g. microscopy)</th>
<th>Rapid/culture independent tests</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>Gold standard</td>
<td>Low to high</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>High</td>
<td>Low to high, almost always different</td>
</tr>
<tr>
<td><strong>Interpretation of positive findings</strong></td>
<td>Usually straightforward</td>
<td>Significant issues</td>
</tr>
<tr>
<td><strong>Range of pathogens detected</strong></td>
<td>All pathogens allowed by growth or test conditions</td>
<td>Limited to specific pathogen tested</td>
</tr>
<tr>
<td><strong>Allows for susceptibility testing &amp; genotyping?</strong></td>
<td>Yes</td>
<td>Generally no</td>
</tr>
</tbody>
</table>
Demise of GC Culture

Rapid NAA test:

• Fast (hours)
• Urine specimen (vs urethral swab)
• Includes *Chlamydia trachomatis*
• High sensitivity
• No susceptibility data
• Specimen incompatible with culture
• Expensive
• Some concerns about false positives
Medical reasons for laboratory testing

- Appropriate treatment
- Prevent unnecessary treatment or procedures
Public health reasons for surveillance / outbreak investigation

- Limit transmission
- Control underlying problems
- Monitor trends → informed policy development
Impacts

- Patient Management
- Public Health Programs
  - Requiring accurate case counts
  - Isolate-requiring
RIDT (+) for Flu B

Interpretation: Influenza B virus infection likely

- Treat with antiviral agents, if appropriate.
- Consider whether additional diagnostic testing and/or empiric antibiotic therapy for co-infections is indicated.

* For guidance on antiviral treatment see: CDC/ACIP/IDSA

RIDT (+) for Flu A

Interpretation: Influenza A virus infection likely. Could be novel H1N1, seasonal H1N1, H3N2, or, rarely, an influenza A virus of animal origin

- Treat with antiviral agents, if appropriate.
- Consider whether additional diagnostic testing to determine influenza A subtype and/or if empiric antibiotic therapy for co-infections is indicated.

* For guidance on antiviral treatment see: CDC/ACIP/IDSA

RIDT (-) for Flu A and B

Interpretation: Can not rule out Influenza virus infection

- Use clinical symptoms, severity, and underlying disease to decide if antiviral treatment is appropriate.
- Do NOT use a negative test to send a symptomatic child back to school, to rule out an institutional outbreak, or to dictate infection control measures.
- Consider whether further influenza specific testing using viral culture or rRT-PCR is necessary.
- Consider whether additional diagnostic testing and/or empiric antibiotic therapy for co-infections is indicated.

* For guidance on antiviral treatment see: CDC/ACIP/IDSA
Recommendations for Diagnosis of Shiga Toxin--Producing *Escherichia coli* Infections by Clinical Laboratories

Importance of Culture Confirmation of Shiga Toxin-producing *Escherichia coli* Infection as Illustrated by Outbreaks of Gastroenteritis --- New York and North Carolina, 2005

*Escherichia coli* O157:H7 and other strains of *E. coli* that produce Shiga toxin are collectively known as Shiga toxin-producing *E. coli* (STEC). The current outbreak of STEC O157 infections associated with eating fresh spinach illustrates the importance of obtaining isolates to identify the source of the infections. Laboratory methods that do not require bacterial culture of stool specimens to identify STEC are being used increasingly by clinical diagnostic laboratories, sometimes without subsequent confirmation of a strain by isolating it in culture. This report describes findings from outbreaks of gastroenteritis in 2005 in New York and North Carolina in which clinical diagnostic laboratories initially used only non-culture methods to detect Shiga toxin (Stx). The findings highlight the importance of confirmation of Stx-positive stool specimens by bacterial culture for timely and reliable identification of STEC infections, including *E. coli* O157 and non-O157 STEC, to enable implementation of appropriate public health actions. An important part of this identification is...
Impacts

- Patient Management
- Public Health Programs
  - Requiring accurate case counts
    - Burden
    - Attribution
    - Trends
  - Isolate-requiring
Estimates of Foodborne Illness
Variability in diagnostic test performance

Incidence of STEC infections in FoodNet, 2008–2011

2011: 60 (36%) of 184 broths sent public health laboratories could be confirmed as Shiga toxin-positive

- Culture-confirmed STEC + all additional Shiga toxin-positive broths
- Culture-confirmed STEC + additional Shiga toxin-positive broths confirmed at Public Health Lab
- All culture-confirmed STEC
Impacts

- Patient Management
- Public Health Programs
  - Requiring accurate case counts
  - **Isolate-requiring**
    - Subtype-based tracking programs
    - Susceptibility monitoring
    - Subtype-based attribution studies
# Selected Microbial Disease Agents Under Surveillance

<table>
<thead>
<tr>
<th>Agent</th>
<th>Public health surveillance</th>
<th>Isolate significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella spp.</em></td>
<td>Subtype, AST</td>
<td>++++</td>
</tr>
<tr>
<td>Shigatoxin-producing <em>E. coli</em></td>
<td>Subtype, AST</td>
<td>++++</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Subtype, AST</td>
<td>++++</td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>Genotype, AST</td>
<td>++++</td>
</tr>
<tr>
<td><em>Bordetella pertussis</em></td>
<td>AST</td>
<td>+++</td>
</tr>
<tr>
<td><em>Neisseria meningitidis</em></td>
<td>Subtype, AST</td>
<td>+++</td>
</tr>
<tr>
<td><em>Legionella pneumophila</em></td>
<td>Subtype (outbreaks)</td>
<td>++</td>
</tr>
<tr>
<td>Influenza virus</td>
<td>Serotype, AST</td>
<td>++</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoea</em></td>
<td>AST</td>
<td>+</td>
</tr>
<tr>
<td>Methicillin-resistant <em>Staphylococcus aureus</em></td>
<td>Subtype (outbreaks)</td>
<td>+</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>AST</td>
<td>?</td>
</tr>
</tbody>
</table>
Global Meat Trade

LEGEND
- **Pork**
- **Beef**
- **Poultry**

Source: Center for Global Food Issues
Nationwide reporting began in 1912
Reported *Salmonella* infections in the United States, 1920-2006

Typhoid Fever  Non-typhoid Salmonellosis

NATIONAL SALMONELLA SEROTYPE SURVEILLANCE

CDC, National surveillance data
Salmonellosis Cases
Minnesota; June – October 1994

Salmonella spp.  Salmonella Enteritidis
Nationwide reporting began in 1912
Reported *Salmonella* infections in the United States, 1920-2006

Incidence per 100,000 population

- **Typhoid Fever**
- **Non-typhoid Salmonellosis**

National salmonella serotype surveillance

CDC, National surveillance data
87 labs in the PulseNet USA network

- CDC PulseNet headquarters
- Regional labs
- Local and secondary state labs
- Federal labs

December 2011
PulseNet/VetNet Electronic Communication

State and Local Public health laboratories

State Departments of Agriculture

PFGE patterns (~50,000/yr to PulseNet)

National databases

USDA  E. coli pathogens

Salmonella sp. and Campylobacter spp.

VetNet
Pathogen Specific Surveillance

Case reports

Clinical Microbiology

Public Health

Case interviews

Prevention / control activities
Exploiting the True Potential of Agent-Based Surveillance

Informational trace-backs

Enhanced exposure-gathering, laboratory, communication, and analysis methods
“A Big Victory for Public Health”

FDA decision to withdraw the use of Baytril in poultry

In a landmark decision, U.S. Food and Drug Administration (FDA) recently ordered the withdrawal of Baytril, an enrofloxacin, from use in poultry. This decision was reached after years of opposition from the pharmaceutical industry and public health advocates. Public health experts, including those in the National Antimicrobial Resistance Monitoring System (NARMS), have long been concerned about the use of antimicrobials in poultry. The FDA's ruling is seen as a significant victory for public health as it is expected to reduce the development of antibiotic-resistant bacteria in poultry and, consequently, in humans who consume poultry products.

Please refer to the letter on the reverse side of this page for more information.

FoodNet publications that provided the scientific basis for the ruling include:

83 member countries from 7 national and regional PulseNet networks

PulseNet
- Canada
- USA
- Latin America & Caribbean

PulseNet
- Europe
- Africa
- Middle East
- Asia Pacific
Current PulseNet Methods (PFGE and MLVA) are Isolate-Dependent

(Note: so is whole genome sequencing, and most other methods being considered)
EHEC O104:H4

The current EHEC O104:H4, causing a severe outbreak in Germany (May 2011), is microbiologically characterized as follows (1) (27.5.2011; updated 01.6.2011):

- Shigatoxin 1: + (negative)
- Shigatoxin 2 (vtx2a): + (positive)
- Intimin (eae): + (negative)
- Enterohemolysin: - (negative)

EaggEC virulence plasmid:
- aatA-PCR: + (positive) (ABC-transporter protein gene)
- aggR-PCR: + (positive) (master regulator gene of Vir-plasmid genes)
- aap-PCR: + (positive) (secreted protein dispersin gene)
- aggA-PCR: + (positive) (AAF/I-fimbral subunit-gene)
- aggC-PCR: + (positive) (AAF/I-fimbral operon-gene)

MLST Sequence Type:

ST678 (adk 6, fumC6, gyrB 5, icd 136, mdh 9, purA 7, recA 7). (**)
Crisis

Danger + Opportunity
Successful Adaptation to Culture Demise

Sexually Transmitted Diseases (STDs)

Gonococcal Isolate Surveillance Project

The Gonococcal Isolate Surveillance Project (GISP) was established in 1986 to monitor trends in antimicrobial susceptibilities of strains of N. gonorrhoeae in the United States. The project collects isolates from patients diagnosed with gonorrhea at selected sites and tests them for antimicrobial susceptibility. The results are reported to the CDC, which publishes the findings in the weekly Morbidity and Mortality Weekly Report (MMWR). The project also provides feedback to healthcare providers and public health officials about the emergence of resistant strains of N. gonorrhoeae.

Figure 6. Age distribution of GISP participants and nationally reported gonorrhea cases in men, 2006
General Strategies to Address Issue

- **Short-term**: Preserve isolates
- **Longer-term**: Develop culture-independent pathogen characterization methods
- **Very long-term**: paradigm shifting technologies
Tomorrow’s Breakout Sessions

- **Short term**
  - A. Regulatory and Device Industry Strategies
  - B. Clinical and Public Health Practice
  - C. Strategies (1) including culture-independent pathogen characterization assay development
  - D. Strategies (2) same as (1) plus susceptibility surveillance

- **Longer term**
  - E. Novel approaches, known but unproven technologies or processes

- **Very long term**
Reflex Culture

Follow-up culture automatically initiated when positive culture independent-based laboratory test results are observed.

(possible when the specimen collected is compatible with culture)
Short-term: Preserve isolates

- Work with medical industry to make new tests compatible with public health needs
- Change criteria for medical device licensure?
- Make reflex culture reimbursable?
- Modify State reporting rules
- Develop isolate recovery capacity for PHLs
- Sentinel culture-based surveillance?
Longer-term: Develop culture-independent pathogen characterization methods

- Identify ID/subtype/virulence targets for direct molecular detection
- New testing strategies (Point-of-care? Public health labs? Reference labs?)
- Develop global consensus
# Bacteria in Human Stools

Up to $10^{12}$ bacteria/ml; ~500 species

<table>
<thead>
<tr>
<th>Bacteria (Genus and Species)</th>
<th>Bacteria (Genus and Species)</th>
<th>Bacteria (Genus and Species)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides fragilis</td>
<td>Clostridium putrificum</td>
<td>Streptococcus sp. (S. equinus)</td>
</tr>
<tr>
<td>Bacteroides vulgatus</td>
<td>Clostridium sp. (C. cadaveris)</td>
<td>Streptococcus sp. (S. pyogenes)</td>
</tr>
<tr>
<td>Bacteroides eggerthii</td>
<td>Clostridium difficile</td>
<td>Enterococcus faecalis</td>
</tr>
<tr>
<td>Bacteroides sp. (B. fragilis)</td>
<td>Eubacterium tenue</td>
<td>Enterococcus gallinarum</td>
</tr>
<tr>
<td>Bacteroides sp. (B. thetaotaomicron)</td>
<td>Clostridium bifermentans</td>
<td>Lactobacillus acidophilus</td>
</tr>
<tr>
<td>Bacteroides sp. (B. vulgatus)</td>
<td>Clostridium sp. (C. sordellii)</td>
<td>Weissella kandleri</td>
</tr>
<tr>
<td>Bacteroides sp. (B. eggerthii)</td>
<td>Peptostreptococcus (P. anaerobius)</td>
<td>Lactobacillus fermentum</td>
</tr>
<tr>
<td>Bacteroides sp. (B. uniformis)</td>
<td>Fusobacterium nucleatumd</td>
<td>Vagococcus fluvialis</td>
</tr>
<tr>
<td>Cytophaga xylanolytica</td>
<td>Eubacterium plautii</td>
<td>Bifidobacterium infantis</td>
</tr>
<tr>
<td>Bacteroides distasonis</td>
<td>Eubacterium sp. (E. cylindroides)</td>
<td>Bifidobacterium dentium</td>
</tr>
<tr>
<td>Bacteroides sp. (B. distasonis)</td>
<td>Streptococcus sanguis</td>
<td>Bifidobacterium sp. (B. longum)</td>
</tr>
<tr>
<td>Clostridium oroticum</td>
<td>Clostridium sp. (S. mitis)</td>
<td>Bifidobacterium adolescentis</td>
</tr>
<tr>
<td>Clostridium sp. (C. nexile)</td>
<td>Streptococcus oralis</td>
<td>Bifidobacterium pseudolongum</td>
</tr>
<tr>
<td>Ruminococcus hansenii</td>
<td>Streptococcus intermedius</td>
<td>Escherichia coli</td>
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<tr>
<td>Ruminococcus productus</td>
<td>Lactococcus lactis subsp. cremoris</td>
<td>Carnobacterium divergens</td>
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<td>Eubacterium ventriosum</td>
<td>Streptococcus sp. (S. mitis)</td>
<td>Lactobacillus maltaromicus</td>
</tr>
<tr>
<td>Clostridium sp. (C. clostriiforme)</td>
<td>Streptococcus sp. (S. bovis)</td>
<td>Salmonella sp. (S. typhi)</td>
</tr>
<tr>
<td>Clostridium histolyticum</td>
<td>Streptococcus sp. (S. equi subsp. equi)</td>
<td>Enterobacter sp. (E. aerogenes)</td>
</tr>
<tr>
<td>Clostridium sp. (C. beijerinckii)</td>
<td>Streptococcus mutans</td>
<td>Serratia sp. (S. marcescens)</td>
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<tr>
<td>Clostridium sp. (C. butyricum)</td>
<td>Streptococcus sp. (S. sanguis)</td>
<td>Proteus sp. (P. vulgaris)</td>
</tr>
<tr>
<td>Clostridium sp. (C. perfringens)</td>
<td>Streptococcus sp. (S. salivarius)</td>
<td>Klebsiella sp. (K. pneumoniae)</td>
</tr>
</tbody>
</table>
Culture-Independent Subtyping/Virulence Assays: Targets

Conserved primer sites

![Diagram of conserved primer sites and variable region]

Variable primer site

Strain/species
- A
- B
- C

Scoring

- **Allele**
  - Different sequence
  - Different size (MLVA)

- **Presence/Absence**
  - Amplicon produced
  - No amplicon
Opportunities

- Faster results (better exposure recall, faster intervention)
- Wider understanding of disease causation
- Opportunity for global consensus on new methods
Opportunity for Global Consensus

83 member countries from 7 national and regional PulseNet networks

December 2011
The Surveillance Process
Laboratory Reporting Takes Time

- Patient Eats Contaminated Food: 1 – 3 days
- Stool Sample Collected
- Public Health Laboratory Receives Sample
- Patient Becomes Ill
- Contact with health care system: 1 – 5 days
- Diagnosis: 1 – 3 days
- Shipping: 0 – 7 days
- Serotyping & DNA fingerprinting: 2 – 10 days
- Salmonella Identified
- Case Confirmed as Part of Outbreak
Cladistic Methods --→ More Flexible Case Definitions
## Virulence Factors in Shiga toxin-producing *E. coli* (STEC)

<table>
<thead>
<tr>
<th>Virulence factor</th>
<th>Gene Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shiga toxin (<em>stx</em>)</td>
<td>Phage</td>
</tr>
<tr>
<td>Intimin (<em>eae</em>)</td>
<td>PAI (LEE)</td>
</tr>
<tr>
<td>Enterohemolysin (EhxA, HlyA)</td>
<td>Plasmid (pO157)</td>
</tr>
<tr>
<td>Non-LEE effectors (<em>nle</em>)</td>
<td>PAI’s</td>
</tr>
<tr>
<td><em>Saa</em> adhesin <em>(STEC autoagglutinating adhesin)</em></td>
<td>Plasmid</td>
</tr>
<tr>
<td>Subtilase</td>
<td>Plasmid</td>
</tr>
<tr>
<td>More...........</td>
<td></td>
</tr>
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</table>

Courtesy of P. Gerner-Smidt
<table>
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<tr>
<th>O26</th>
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<td>LEE Z5110 eae</td>
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<td>OI-122 Z4326 Mod 2</td>
<td>OI-122 Z4332 Mod 3</td>
<td>OI-122 Z4333 Mod 3</td>
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<td>katP</td>
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</table>

**STEC Detection and Genotyping Assay**

**Targets and timeline for assay development**

- **Summer 2011**
- **Fall 2011**
- **Fall 2012**
Culture-Independent Subtyping/Virulence Assays: Potential Issues

- Sensitivity
- Different assays needed for each pathogen?
- Suitable targets may not exist for all pathogens
GISP-like sentinel surveillance?

Combination of molecular screening and sentinel surveillance?
Very Long-term: Paradigm-shifting Technologies or Processes

- Single cell isolation and sequencing; mass MS screening, others?
- Metagenomics
- Other ways of conducting surveillance and outbreak detection?
Metagenomic Approach

- Sequence selected targets (e.g. 16S, 18S rRNA)
- “Deep sequencing” (all genetic material in sample)
  - Assemble and identify contigs
  - Extract and analyze sequences of interest
Etiology of Acute Gastroenteritis in the U.S.

Total Cases
- unknown: 82%
- known agents: 18%

Known Etiology
- viruses: 67%
- bacteria: 30%
- parasites: 3%
- unknown: 82%
What is Being Done?

- This Meeting
- Workgroups (CDC, APHL, CSTE)
- CDC Genomics project
- DTRA/DOD/CDC Metagenomics project
- Much more needed
High probability, high impact issue

Risks of inaction and benefits of change are significant
<table>
<thead>
<tr>
<th>Marc Allard</th>
<th>Patricia Griffin</th>
<th>Michele Parsons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robyn Atkinson</td>
<td>Thomas Hammack</td>
<td>Efrain Ribot</td>
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<td>John Besser</td>
<td>Vincent Hill</td>
<td>Shari Shea</td>
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<td>Cheryl Bopp</td>
<td>Kristin Holt</td>
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<td>Brenda Brown</td>
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<td>Collette Fitzgerald</td>
<td>Beth McGlinchey</td>
<td>Jean Whichard</td>
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<tr>
<td>Peter Gerner-Smidt</td>
<td>Stephen Morse</td>
<td>Ian Williams</td>
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