Culture-Independent Diagnostics Testing: The End of Surveillance for Foodborne Infections?

John Besser, Ph.D
Deputy Chief, Enteric Diseases Laboratory Branch
CDC/NCEZID/DFBMD

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.
Why Track Foodborne Disease?

- Monitor trends in disease over time
- Monitor foods causing illnesses
- Detect outbreaks
Bacterial Culture
Rapid ("Culture-Independent"; "Non-Culture") Tests
Luminex Receives FDA Clearance for First Comprehensive Gastrointestinal Pathogen Infectious Disease Diagnostic in the United States

xTAG® Gastrointestinal Pathogen Panel (GPP) simultaneously tests for greater than 90% of bacterial, viral, and parasitic causes of infectious gastroenteritis in a single molecular test.

RELATED QUOTES

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Price</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMNX</td>
<td>18.17</td>
<td>0.36</td>
</tr>
</tbody>
</table>

AUSTIN, Texas, Jan. 15, 2013 /PRNewswire/ -- Luminex Corporation (LMNX) announced today that it has received FDA clearance for its xTAG Gastrointestinal Pathogen Panel (GPP), the first comprehensive molecular diagnostic assay that tests for greater than 90% of bacterial, viral, and parasitic causes of infectious gastroenteritis in a single assay. The xTAG GPP assay can be an important clinical tool in the management of
New diagnostic tests for common foodborne pathogens such as *Salmonella*, *Campylobacter*, and *Escherichia coli* may hinder the ability of public health officials to detect multistate outbreaks. The problem is an inability to trace contamination to its source.
## Rapid / Culture-Independent Tests versus Culture

<table>
<thead>
<tr>
<th>Feature</th>
<th>Culture</th>
<th>Rapid/culture-independent tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed</td>
<td>Slow</td>
<td>Fast</td>
</tr>
<tr>
<td>Infrastructure needed</td>
<td>Significant</td>
<td>Minimal</td>
</tr>
<tr>
<td>Expertise required</td>
<td>Significant</td>
<td>Minimal</td>
</tr>
<tr>
<td>Labor cost</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Cost of materials</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>Culture or standard tests (e.g. microscopy)</td>
<td>Rapid/culture independent tests</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>--------------------------------------------</td>
<td>---------------------------------</td>
</tr>
<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; may include unculturable/difficult to culture pathogens</td>
</tr>
<tr>
<td>Allows for susceptibility testing &amp; genotyping?</td>
<td>Yes</td>
<td>Generally no</td>
</tr>
</tbody>
</table>
Now you can test for 15 key bacteria, viruses, and parasites — *all in under 5 hours*
The “Cloud” of Stool Testing Methods

Send outs:
- Noro PCR
- O & P
- Bacterial isolates to State

C. difficile toxin PCR

Giardia/Crypto EIA

Specimen arrives in Lab

Order entry and correction

Primary Set-Up
- Enteric culture inoculation
- Specimen aliquoting

Culture desk
(Salmonella, Shigella, Campylobacter, STEC, Yersinia, Vibrio)

Virology shell culture
- Rotavirus EIA
- DFA confirmatory Giardia/Crypto

Specimen arrives in Lab

Specimen arrives in Lab

Send outs:
- Noro PCR
- O & P
- Bacterial isolates to State

C. difficile toxin PCR

Giardia/Crypto EIA

Adapted from K. Chapin, 2013
Stool Testing with PCR Panels

1. Specimen arrives in Lab
2. Order entry and correction
3. PCR multi-agent panel(s)
   • Specimen to State
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>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella spp.</em></td>
<td>Subtype, AST</td>
</tr>
<tr>
<td>Shigatoxin-producing <em>E. coli</em></td>
<td>Subtype, AST</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Subtype, AST</td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>Genotype, AST</td>
</tr>
<tr>
<td><em>Bordetella pertussis</em></td>
<td>AST</td>
</tr>
<tr>
<td><em>Neisseria meningitidis</em></td>
<td>Subtype, AST</td>
</tr>
<tr>
<td><em>Legionella pneumophila</em></td>
<td>Subtype (outbreaks)</td>
</tr>
<tr>
<td>Influenza virus</td>
<td>Serotype, AST</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoea</em></td>
<td>AST</td>
</tr>
<tr>
<td>Methicillin-resistant <em>Staphylococcus aureus</em></td>
<td>Subtype (outbreaks)</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>AST</td>
</tr>
</tbody>
</table>
Nationwide reporting began in 1912.

Reported *Salmonella* infections in the United States, 1920-2006

CDC, National surveillance data
Ice-Cream Associated Outbreak; U.S.A., 1994

>250,000 cases
48 States

![Graph showing the incidence of Typhoid Fever and Non-typhoid Salmonellosis from 1920 to 2000.](image)

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

**National salmonella serotype surveillance**

CDC, National surveillance data
The decade's 10 biggest food-borne illness outbreaks

By Jacque Wilson, CNN
updated 11:04 AM EST, Fri September 30

Peanut butter

Eggs

Peppers

Tomatoes

Ground turkey

Deli meats

Roma tomatoes

Spinach
2013 PulseNet-Triggered or Enhanced Outbreak Investigations (so far)

UPDATE 1-Salmonella linked to ground beef sickens 16 -CDC
January 28, 2013 | Reuters
By Eric M. Johnson

Multistate Salmonella Heidelberg Outbreak Linked To Foster Farms Chicken Increases To 128 Cases
With four new cases of Salmonella Heidelberg reported from Alabama (1), California (3), and Washington (1), the total number of cases in the multistate outbreak now stands at 128. Centers for Disease Control and Prevention (CDC) said.

Three MN Cases Added to Salmonella Outbreak from Live Poultry
Three cases of Salmonella infections linked to live poultry have been added to the Minnesota outbreak, bringing the total number of cases to 30, officials said.

10 million pounds of frozen pizza, snacks recalled in rare E. coli outbreak
By Joane Alcvecia, Senior Writer, NBC News
A New York snack food maker now says it is recalling 10 million pounds of frozen pizza, mozzarella bites, and chicken parts and other products linked to a rare and potentially dangerous outbreak of E. coli.

Salmonella outbreak linked to cucumbers sickens 73
By Rachael Retheby / Published April 25, 2013 / MyHealthNewsDaily

Deadly Listeria Outbreak May Be Linked to Cheese
Grave Brothers has recalled several cheeses, including the Les Freres, after a Listeria outbreak.
### Largest U.S. Food Recalls in which PulseNet Played a Prominent Role

<table>
<thead>
<tr>
<th>Year</th>
<th>Pathogen</th>
<th>Food</th>
<th>Amount recalled</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td><em>Listeria monocytogenes</em></td>
<td>Cantaloupe</td>
<td>Unknown</td>
</tr>
<tr>
<td>2011</td>
<td><em>Salmonella</em> Heidelberg</td>
<td>Ground turkey products</td>
<td>&gt;36,000,000 lbs</td>
</tr>
<tr>
<td>2010</td>
<td><em>Salmonella Enteritidis</em></td>
<td>Shell eggs</td>
<td>&gt;500,000,000 eggs</td>
</tr>
<tr>
<td>2010</td>
<td><em>Salmonella</em> Montevideo</td>
<td>Ready-to-eat Italian sausage products/pepper</td>
<td>&gt;1,263,754 lbs</td>
</tr>
<tr>
<td>2009</td>
<td><em>E. coli</em> O157:H7</td>
<td>Cookie dough</td>
<td>300,000 cases of product</td>
</tr>
<tr>
<td>2009</td>
<td><em>Salmonella Typhimurium</em></td>
<td>Peanut butter/peanut products</td>
<td>&gt;3000 types of products</td>
</tr>
<tr>
<td>2008</td>
<td><em>E. coli</em> O157:H7</td>
<td>Ground beef</td>
<td>5,300,000 lbs</td>
</tr>
<tr>
<td>2007</td>
<td><em>Salmonella</em> I 4,5,12:i:-</td>
<td>Frozen pot pies</td>
<td>Millions of pot pies</td>
</tr>
<tr>
<td>2007</td>
<td><em>E. coli</em> O157:H7</td>
<td>Frozen pizza</td>
<td>5,000,000 pizzas</td>
</tr>
<tr>
<td>2007</td>
<td><em>E. coli</em> O157:H7</td>
<td>Ground beef (3 outbreaks)</td>
<td>35,400,000 lbs</td>
</tr>
<tr>
<td>2006</td>
<td><em>Salmonella</em> Tennessee</td>
<td>Peanut butter</td>
<td>326,000,000 lbs</td>
</tr>
<tr>
<td>2004</td>
<td><em>Salmonella</em> Enteritidis</td>
<td>Raw almonds</td>
<td>13,000,000 lbs</td>
</tr>
<tr>
<td>2003/'09</td>
<td><em>E. coli</em> O157:H7</td>
<td>Blade Tenderized Frozen Steak</td>
<td>865,046 lbs</td>
</tr>
<tr>
<td>2002</td>
<td><em>Listeria monocytogenes</em></td>
<td>Ready-to-eat poultry products</td>
<td>27,400,000 lbs</td>
</tr>
<tr>
<td>2002</td>
<td><em>E. coli</em> O157:H7</td>
<td>Ground beef</td>
<td>18,600,000 lbs</td>
</tr>
<tr>
<td>2000</td>
<td><em>Listeria monocytogenes</em></td>
<td>Ready-to-eat poultry products</td>
<td>16,900,000 lbs</td>
</tr>
<tr>
<td>2000</td>
<td><em>E. coli</em> O157:H7</td>
<td>Ground beef</td>
<td>1,100,000 lbs</td>
</tr>
<tr>
<td>1998</td>
<td><em>Listeria monocytogenes</em></td>
<td>Hot dogs, deli meats</td>
<td>35,000,000 lbs</td>
</tr>
<tr>
<td>1998/'08</td>
<td><em>Salmonella</em> Agona</td>
<td>Toasted oats cereal</td>
<td>&gt;3,000,000 lbs</td>
</tr>
<tr>
<td>1997</td>
<td><em>E. coli</em> O157:H7</td>
<td>Frozen ground beef</td>
<td>25,000,000 lbs</td>
</tr>
</tbody>
</table>
Industries Stimulated to Change by PulseNet-Triggered Investigations

- Ready-to-eat & “ready-to cook” foods
  - Peanut products
  - Leafy greens
  - Sprouts
  - Dog food
  - Eggs
  - Tree nuts
  - Poultry
  - Spices
  - Chickens
  - Mellons
Under-appreciated Sources of Infection

- *Salmonella* in reptiles and amphibians
- *Salmonella* in baby chicks and ducks
- *Vibrio vulnificus* after fish handling
- *Salmonella* in "feeder" mice
- *Salmonella* from microbiology lab exposure
- Multiple pathogens from live bird markets
- *Salmonella* in owl pellets
- STEC in petting zoos
- *Salmonella* and "pocket pets"
Nationwide reporting began in 1912
Reported *Salmonella* infections in the United States, 1920-2006

*Typhoid Fever*  *Non-typhoid Salmonellosis*

CDC, National surveillance data
### Number of cases reported to the WHO as for 21 July 2011

<table>
<thead>
<tr>
<th>Country</th>
<th>Deaths</th>
<th>HUS cases</th>
<th>Non-HUS cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Canada</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Denmark</td>
<td>0</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>France</td>
<td>0</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Germany</td>
<td>48</td>
<td>857</td>
<td>3078</td>
</tr>
<tr>
<td>Greece</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Luxembourg</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Netherlands</td>
<td>0</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Norway</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Poland</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Spain</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sweden</td>
<td>1</td>
<td>18</td>
<td>35</td>
</tr>
<tr>
<td>Switzerland</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>0</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>United States</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>50</td>
<td>908</td>
<td>3,167</td>
</tr>
</tbody>
</table>

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**World health officials scramble to stem deadly E. coli outbreak**

By the CNN Wire Staff
June 3, 2011, 4:13 am, EDT

Tomatoes and cucumbers are displayed on May 30, 2011, in a stall in a supermarket in Paris.

**STORY HIGHLIGHTS**

- **NEW** Spain's produce "safe for all consumers," Spanish official says
- **(CNN)** -- Infectious disease detectives worldwide rushed Thursday to find the cause of an outbreak of a rare strain of E. coli that has spread to 10 countries and is blamed for at least 16 deaths and hundreds of illnesses.
“It is ironic that the licensure of new tests that are arguably better for the patient and testing laboratory may result in degredation of the safety of our food supply, and many thousands of additional cases”
Culture-Independent Diagnostics Testing: The End of Surveillance for Foodborne Infections?

What Are the Options?
General Strategies to Address Issue

- **Short-term**: Make sure isolates continue to be available for public health activities

- **Long-term**: Develop culture-independent pathogen characterization methods
Strategies to Assure Continuing Specimens and Isolates for Public Health Through...

- Collaboration with medical device industry
- Regulatory (device licensure, laboratory regulation) authority
- Reimbursement (of clinical laboratories for reflex culture)
General Strategies to Address Issue

- **Short-term**: Make sure isolates continue to be available for public health activities

- **Long-term**: Develop culture-independent pathogen characterization methods
Diagnostic Test

Pathogen-specific targets

- Bacteroides, *spp*
- Clostridium, *spp*
- Bifidobacterium, *spp*
- Hundreds of other *spp*

Conserved within, and unique to pathogen of interest

- *E. coli* O157:H7 strain 1
- *E. coli* O157:H7 strain 2
- Low virulence STEC
- Other *E. coli*
### Public Health Characterization

<table>
<thead>
<tr>
<th>Pathogen-specific targets</th>
<th>Resistance markers</th>
<th>Virulence determinants</th>
<th>Strain markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Bacteriodes, spp$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Clostridium, spp$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Bifidobacterium, spp$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hundreds of other $spp$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E. coli$ O157:H7 strain 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E. coli$ O157:H7 strain 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low virulence STEC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other $E. coli$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Public Health Characterization

Pathogen-specific targets

Resistance markers

Virulence determinants

Strain markers

Establish linkage

Bacteriodes, spp

Clostridium, spp

Bifidobacterium, spp

Hundreds of other spp

E. coli O157:H7 strain 1

E. coli O157:H7 strain 2

Low virulence STEC

Other E. coli
Impact of CIDTs on Surveillance Timeline

1 – 3 days

Contact with health care system: 1 – 5 days

Diagnosis: 1 – 3 days

Shipping: 0 – 7 days

Sero_typing & DNA fingerprinting: 2 – 10 days

Case Confirmed as Part of Outbreak

Patient Eats Contaminated Food

Stool Sample Collected

Public Health Laboratory Receives Sample

Patient Becomes Ill

Salmonella Identified

CDC

1 – 3 days
Finely Tuning Case Definitions; More accurately Aligning with Food/Environmental Findings

MLVA – *Salmonella* Enteritidis

From Boxrud et al; 2010
Etiology of Acute Gastroenteritis in the U.S.

- Unspecified agents: 80%
- Viruses: 12%
- Bacteria: 8%
- Parasites: 0%

Calculated from:
Advantages of Public Health CIDTs: Better Understanding of Disease

- Pathogen discovery
- Microbial interactions
- Host factors
- Exposures
Laboratory Strategy to Meet The Challenge of Culture
Independent Diagnostic Methods (CIDT)

1. **Preserve cultures**
   - Surveillance by current methods (serotyping, AST, PFGE, MLVA etc.)

2. **Prepare for the future working on pure cultures**
   - 250 STEC genomes study
   - Bigs.db
   - 100k Foodborne pathogens sequencing study
   - LRN metagenomics study

3. **Metagenomics No cultures**
   - 250 STEC genomes study
   - Bigs.db
   - 100k Foodborne pathogens sequencing study
   - LRN metagenomics study
   - Applied Maths, DNASTAR
   - Real-time WGS surveillance of listeriosis
   - Global Microbial Identifier (GMI)

**Surveillance and diagnostics by metagenomics**

**Surveillance by whole genome sequencing (WGS)**

**CIDT Working groups:**
1. Overarching WG
2. Regulatory WG
3. PHL/Funding/Best Practices WG
4. CIDT panel evaluation WG
5. Industry WG

**Communication:**
- White papers, Meetings
- Presentations, Publications

**CIDT Working groups:**
- 1. Overarching WG
- 2. Regulatory WG
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- 4. CIDT panel evaluation WG
- 5. Industry WG

**Surveillance by current methods (serotyping, AST, PFGE, MLVA etc.)**

**Surveillance by whole genome sequencing (WGS)**

**Surveillance and diagnostics by metagenomics**
Culture-Independent Diagnostics Testing: The End of Surveillance for Foodborne Infections?