Culture-Independent Diagnostic Testing: Implications for Public Health

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Collaborative Food Safety Forum
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Major Foodborne Illness Surveillance Systems

Major Categories

I. National case surveillance
II. Sentinel site case surveillance
III. Outbreaks
Estimates of Foodborne Illness

How Safe Is Our Food?

Foodborne Illness Acquired in the United States—Major Pathogens

Foodborne Illness Acquired in the United States—Unspecified Agents

Foodborne Diseases are on the rise in the United States. In recent years, cases have increased in number, severity, and geographic distribution. Although these diseases are common globally, this growing trend is a cause for concern, as it can have serious health and economic implications. In this special issue of Emerging Infectious Diseases, experts provide an overview of the current state of foodborne diseases and discuss strategies to prevent and control them. The articles highlight the importance of surveillance, research, and public education in managing this growing public health threat.
More than 75 labs in the PulseNet network
The decade's 10 biggest food-borne illness outbreaks

By Jacque Wilson, CNN
updated 11:04 AM EST, Fri September 30
PulseNet International in 82 Countries
"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 Baytril, an enrofloxacin, from use in poultry. This decision was made in opposition from the pharmaceutical industry’s interests. Public health contributions from the National Antimicrobial Resistance Monitoring System (NARMS) showed convincing links between human health and antibiotic use in poultry. The decision was ruling a "big victory for public health."

Please refer to the letter on the reverse side of this page.
Bacterial Culture

PulseNet New Zealand
Rapid Tests

Premier™
Premier™ EHEC delivers superior performance and confidence in results to make a timelier and appropriate patient management decision for improved outcomes. Since 1994, Premier™ EHEC has been the most widely utilized and trusted test for the detection of Shiga toxin-producing E. coli.

Premier™ CAMPY
Premier™ CAMPY delivers optimized detection for Campylobacter testing that provides the confidence in results to make more timely and appropriate patient management decisions for improved outcomes.

ImmunoCard STAT!®
ImmunoCard STAT!® EHEC is the first rapid, lateral flow immunoassay for the clinical market that detects all Shiga toxin-producing E. coli. ImmunoCard STAT!® EHEC differentiates between Shiga 1 and Shiga 2.

ImmunoCard STAT!® E. coli 0157 Plus

ImmunoCard STAT!® CAMPY
ImmunoCard STAT!® CAMPY provides accurate detection of Campylobacter without the lengthy, labor-intensive procedure associated with culture.

http://www.meridianbioscience.com/diagnostic-products/foodborne
Rapid Tests

NEW xTAG® Gastrointestinal Pathogen Panel (GPP)

The evolution of GI diagnosis

Now you can test for 15 key bacteria, viruses, and parasites – all in under 5 hours

- xTAG® GPP is the first diagnostic to offer detection of 15 major gastrointestinal pathogens in a single test
- Results within 5 hours for timely and better patient care
- Fast turn-around time and multiplex
## Rapid / Culture-Independent Tests versus Culture

<table>
<thead>
<tr>
<th></th>
<th>Rapid/non-culture tests</th>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Speed</strong></td>
<td>Fast</td>
<td>Slow</td>
</tr>
<tr>
<td><strong>Infrastructure needed</strong></td>
<td>Minimal</td>
<td>Significant</td>
</tr>
<tr>
<td><strong>Expertise required</strong></td>
<td>Minimal</td>
<td>Significant</td>
</tr>
<tr>
<td><strong>Labor cost</strong></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td><strong>Cost of materials</strong></td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Comparison</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>Sensitivity</td>
<td>Gold standard</td>
<td>Low to high</td>
</tr>
<tr>
<td>Specificity</td>
<td>High</td>
<td>Low to high, almost always different</td>
</tr>
<tr>
<td>Interpretation of positive findings</td>
<td>Usually straightforward</td>
<td>Significant issues</td>
</tr>
<tr>
<td>Range of pathogens detected</td>
<td>All pathogens allowed by growth or test conditions</td>
<td>Limited to specific pathogen tested</td>
</tr>
<tr>
<td>Allows for susceptibility testing &amp; genotyping?</td>
<td>Yes</td>
<td>Generally no</td>
</tr>
</tbody>
</table>
Demise of GC Culture

- Rapid (hours)
- Urine specimen (vs urethral swab)
- Includes *Chlamydia trachomatis*
- High sensitivity/specificity
- No susceptibility data
- Specimen incompatible with culture
- Expensive
Sexually Transmitted Diseases (STDs)

Gonococcal Isolate Surveillance Project

The Gonococcal Isolate Surveillance Project was established in 1986 to monitor trends in antibiotic susceptibilities of strains of N. gonorrhoeae. It is a component of the CDC’s National sexually transmitted diseases surveillance system. The Project data is collected from 11 sites from across the United States.

Figure 6. Age distribution of GISP participants and nationally reported gonorrhea cases in men, 2006
Luminex launches xTAG™ Respiratory Viral Panel

Luminex Molecular Diagnostics, a division of Luminex Corporation, has launched xTAG Respiratory Viral Panel (RVP), an assay for the detection of multiple viral types and subtypes, including influenza, metapneumovirus and adenovirus. xTAG has been developed in association with a team of leading virologists and infectious disease specialists. The test can assess 12 viral targets at once and provide qualitative results in just few hours. The test has received 510(k) clearance from the US Food and Drug Administration (FDA) and CE mark for sale in Europe.

xTAG RVP was developed using LMD’s Universal Array which operates on the Luminex xMAP® system, a bioassay detection platform that can detect up to 100 different analytes simultaneously. The system uses lasers to read colour-coded microspheres that attach to specific nucleic acid sequences.

A doctor collects a sample containing viruses from a patient’s nasal cavity, throat, sinuses or bronchi. Nucleic acid is taken out from viruses found in the sample. Samples are then placed in a Luminex xMAP® instrument, where beads are read and analysed by lasers. The lasers identify the colour of the bead that is specific to a virus or subtype, and the presence or absence of the labeled primer. If a particular virus is present, the associated software identifies that as positive.

xTAG RVP facilitates physicians to determine whether a patient has cold, flu or another virus and to prescribe an effective treatment. The assay assists doctors in better decision-making in patient management, besides helping in limiting the spread of infection. This test will aid in reducing the overuse of antibiotics, which results into creating antibiotic resistant bacteria or superbugs.
Impacts

- Patient Management
- Public Health Programs

  - Requiring accurate case counts
    - Burden
    - Attribution
    - Trends

  - Isolate-requiring
    - PulseNet / OutbreakNet
    - NARMS
    - Subtype-based attribution studies
Possible Solutions: Burden, Attribution, Trends

- Understand extent of issue
- Study test performance
- Redefine case definitions
1. Do you test stool specimens for *E. coli* O157 and/or for Shiga toxin on site at your laboratory?
   - Yes (skip to Q2)
   - No, if no, to which laboratories do you send specimens for *E. coli* O157 or Shiga toxin testing?
   - _________________________ {Stop, move to the next pathogen}

2. What method(s) does your laboratory currently use to detect *E. coli* O157 and/or Shiga toxin? (select one)
   - a. Only perform culture for *E. coli* O157 on a selective and differential media (for example SMAC, CT-SMAC, CHROMagar O157, etc)
   - b. Only non-culture based test(s), such as PCR, EIA, lateral flow, or other assays to detect Shiga toxin and/or O157 antigen directly on stool samples or on enrichment broths. We are NOT interested in non-culture techniques used to identify the O157 antigen in bacterial isolates
   - c. Both culture for *E. coli* O157 and direct non-culture methods

3. Based on your answers to the question above, which of the following testing criteria best describes the practice for *E. coli* O157 and/or Shiga toxin in your laboratory:

<table>
<thead>
<tr>
<th>Culture</th>
<th>Non-culture</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Never performed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Performed on all stools submitted to our laboratory for routine culture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Automatically performed IF the other type of test is positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Only performed when requested by a clinician</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Preliminary Results Multi-State Campylobacter Diagnostics Study

- A total of 3.1% (87/2772) of specimens were positive by culture

<table>
<thead>
<tr>
<th>Number of culture positive specimens (n=87)</th>
<th>Premier</th>
<th>ProspeCT</th>
<th>ICS</th>
<th>XspeCT</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P(n=56), Neg (n=3*)</td>
</tr>
<tr>
<td>13</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>P(n=4), Neg (n=8*)</td>
</tr>
<tr>
<td>2</td>
<td>Neg</td>
<td>P</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg*</td>
</tr>
<tr>
<td>1</td>
<td>P</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>P</td>
</tr>
<tr>
<td>5</td>
<td>P</td>
<td>P</td>
<td>Neg</td>
<td>Neg</td>
<td>P</td>
</tr>
<tr>
<td>1</td>
<td>Neg</td>
<td>P</td>
<td>Neg</td>
<td>P</td>
<td>P</td>
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<tr>
<td>2</td>
<td>P</td>
<td>P</td>
<td>Neg</td>
<td>P</td>
<td>P</td>
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<tr>
<td>2</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>Neg</td>
<td>P</td>
</tr>
<tr>
<td>1</td>
<td>P</td>
<td>Neg</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
</tbody>
</table>

Nos. of false negatives: 16, 15, 24, 23, 13

- 5/13 PCR negative specimens tested so far in a different Campylobacter-specific PCR assay. All are positive for Campylobacter.
Impacts

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- Public Health Programs
  - Requiring accurate case counts
    - Burden
    - Attribution
    - Trends
  - Isolate-requiring
    - PulseNet / OutbreakNet
    - NARMS
    - Subtype-based attribution studies
Hazards of Inaction
Hazards of Inaction

- Diminished ability to detect or respond to outbreaks
- Significantly reduced pressure on industry to produce safe food
- Less ability to guide regulatory focus
- Less accurate data to determine burden / attribution
Post-culture STEC Surveillance System

Germany; population 81,471,834 (July 2011 est.)
U.S. Sprout-Associated Outbreaks

- >30 detected and investigated in 10 years
- Relatively few cases
- Investigation expertise developed
- Stimulated regulatory focus
3 dead in German E. coli outbreak; more than 400 sick

The food safety news from Germany continues to be disturbing. Below is a translation of a German article, so excuse any inaccuracies.

The killer germ called EHEC now appeared three lives: In Lower Saxony (Diepholz) passed away a woman (83). She was admitted nine days ago because of bloody diarrhea hospitalized and treated in hospital. The laboratory evidence of EHEC infection was positive. The woman died on Saturday. Investigations by the health department in the immediate death Diepholz also ongoing.

Meanwhile it was announced that a woman possibly died (25) to EHEC in Bremen. The young woman had shown the symptoms of EHEC pathogen, such as the Bremen health authority said. The EHEC pathogens had not been demonstrated so laboratory diagnosis. In Schleswig-Holstein, died in a 80-year-old woman infected. Whether the pathogen was the cause of death is still unclear.

In their search for the source of the infections with the dangerous intestinal bacteria EHEC is making the Frankfurt FDA. All 19 previously in the Main metropolis ill have eaten in the same canteen, a Frankfurt-
Potential Solutions

- **Short term:** process changes to preserve isolates
- **Intermediate term:** develop culture-independent, pathogen-specific subtyping/virulence targets
- **Longer-term:** high-tech solutions (e.g. single cell sequencing and/or metagenomics
Potential Benefits of New Approaches

- Less time to cluster detection
- Less time to interview / tracebacks
- Higher proportion of successful investigations
- Some new technology (e.g. metagenomics) will allow:
  - Better understanding of disease causation and microbial interactions
  - Potential for studying host factors
The Surveillance Process
Laboratory Reporting Takes Time

1 – 3 days

Patient Eats Contaminated Food

Contact with health care system: 1 – 5 days

Stool Sample Collected

Diagnosis: 1 – 3 days

Public Health Laboratory Receives Sample

Shipping: 0 – 7 days

Salmonella Identified

Serotyping & DNA fingerprinting: 2 – 10 days

Case Confirmed as Part of Outbreak
The Surveillance Process

Laboratory Reporting Takes Time

- Patient Eats Contaminated Food
- Stool Sample Collected
- Public Health Laboratory Receives Sample
- Contact with health care system: 1 – 5 days
- Diagnosis: 1 – 3 days
- Shipping: 0 – 7 days
- Serotyping & DNA fingerprinting: 2 – 10 days
- Patient Becomes Ill
- Salmonella Identified
- Case Confirmed as Part of Outbreak

1 – 3 days

2 – 10 days

0 – 7 days

1 – 3 days

1 – 5 days
Summary: Culture Independent Diagnostics Impact

- High probability, high impact issue
- Risks of inaction and benefits of change are significant
Adapted from Daryl Cagle, MSNBC:
Bacteria in Human Stools

Up to $10^{11}$ bacteria/ml; ~500 species

- Bacteroides fragilis
- Bacteroides vulgatus
- Bacteroides eggerthii
- Bacteroides sp. (B. fragilis)
- Bacteroides sp. (B. thetaiotaomicron)
- Bacteroides sp. (B. vulgatus)
- Bacteroides sp. (B. eggerthii)
- Bacteroides sp. (B. uniformis)
- Cytophaga xylanolytica
- Bacteroides distasonis
- Bacteroides sp. (B. distasonis)
- Clostridium oroticum
- Clostridium sp. (C. nexile)
- Ruminococcus hansenii
- Ruminococcus productus
- Eubacterium ventriosum
- Clostridium sp. (C. clostridiiforme)
- Clostridium histolyticum
- Clostridium sp. (C. beijerinckii)
- Clostridium sp. (C. butyricum)
- Clostridium sp. (C. perfringens)
- Clostridium putreficium
- Clostridium sp. (C. cadaveris)
- Clostridium difficile
- Eubacterium tenue
- Clostridium bifermentans
- Clostridium sp. (C. sordellii)
- Peptostreptococcus (P. anaerobius)
- Fusobacterium nucleatum
- Eubacterium plautii
- Eubacterium sp. (E. cylindroides)
- Streptococcus sanguis
- Streptococcus oralis
- Streptococcus intermedius
- Lactococcus lactis subsp. cremoris
- Streptococcus sp. (S. mitis)
- Leuconostoc lactis
- Streptococcus sp. (S. bovis)
- Streptococcus sp. (S. equi subsp. equi)
- Streptococcus mutans
- Streptococcus sp. (S. sanguis)
- Streptococcus sp. (S. salivarius)
- Streptococcus sp. (S. equinus)
- Streptococcus sp. (S. pyogenes)
- Enterococcus faecalis
- Enterococcus gallinarum
- Lactobacillus acidophilus
- Weissella kandleri
- Lactobacillus fermentum
- Vagococcus fluvialis
- Bifidobacterium infantis
- Bifidobacterium dentium
- Bifidobacterium sp. (B. longum)
- Bifidobacterium adolescentis
- Bifidobacterium pseudolongum
- Escherichia coli
- Carnobacterium divergens
- Lactobacillus maltaromicus
- Salmonella sp. (S. typhi)
- Enterobacter sp. (E. aerogenes)
- Serratia sp. (S. marcescens)
- Proteus sp. (P. vulgaris)
- Klebsiella sp. (K. pneumoniae)
Random Shotgun Metagenomics

Clinical Sample

Total Host & Microbial NA

Random Amplification & Sequencing
Pan genome

Meta genome

Core genome
Metagenomic Approach

- Sequence all genetic material in sample
- Assemble and identify contigs
- Extract and analyze sequences of interest
Metagenomics: Potential Benefits

- Fast, culture-independent
- More pathogens / combinations of pathogens detected
- Better understanding of microbial interactions
- Potential for understanding host factors