2015 Update in Infectious Diseases: New Tools in Diagnostic Microbiology

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Medical Director, Central Microbiology Lab
Conflicts of Interest

• **Research Support**
  – Merck and Company
  – Immunexpress, Inc.
  – Nanosphere, Inc.
  – BioFire

• **Consultant**
  – Nanosphere, Inc.
Objectives

• Understand changes in diagnostic testing in the Central Microbiology Laboratory
  – Bacteremia
  – Gastroenteritis

• Re-examine the role of molecular testing for diagnosis of *Clostridium difficile*
Trends in Diagnostic Microbiology

- **Blood Culture**
  - MALDI-TOF
  - Verigene BC GP/BC GN
  - BioFire BCID

- **Respiratory Panels**
  - BioFire
  - Verigene
  - Luminex
  - Genmark

- **GI Panels**
  - BioFire GI Panel
  - Verigene Enteric Panel
  - Genmark
  - Luminex
  - BD

- **CSF Panel**
  - BioFire
Methods for Rapid Bacterial Identification in Positive Blood Cultures

**Bottle Culture Incubation**

**Sub-culture**

**Susceptibility Testing**

- **MOLECULAR TESTS**
  - Cepheid Xpert® MRSA/SA
  - PNA-FISH®, Quick FISH™
  - Verigene® BC GP, BC GN
  - BioFire FilmArray™ BCID

- **T2 Biosystems – Candida spp**
- more coming?

- **1-3 hrs**
- **Mins**
- **MALDI* Mins**

*Matrix assisted laser desorption/ionization
What’s All this About?

**Comments:** , at 1609 9/15 gw, ON 9/16/15 AT 0226 EC Critical value not notified because of anticipated critical value

**FINAL Result:** Staphylococcus aureus identified by MALDI TOF Mass Spectrometry No Methicillin resistance detected  
Updated: 09/17/2015 11:03

**Method:** MIC

**Comments:** This test was developed and its performance characteristics determined by the Intermountain Healthcare Central Laboratory. The U.S. Food and Drug Administration has not approved or cleared this test. FDA clearance or approval is not currently required.

**Result:** Abnormal result forwarded to IMC Lab call center
Impact of Bacterial Identification by MALDI with Antimicrobial Stewardship in Patients with Bloodstream Infections

ID BLOOD CULTURE BROTH

- Gram negative bacteremia
- Time to Identification: $47.1 \pm 13.7$ vs. $24.4 \pm 11.4$ (P<0.001)
- Antibiotic
  - Time to adjustment: 75 vs. 29 hrs (P=0.004)
  - Time to active: 73.2 vs. 36.5 hours (P < 0.001)
- Mortality: 5.6% vs. 10.7%, P=0.19

ID FROM ISOLATES

- All bacteremia
- Time to Identification: $84.0$ vs. $55.9$ hours (P<0.001)
- Antibiotic
  - Time to effective: 30.1 vs. 20.4 hrs, P<0.021
  - Time to optimal: 90.3 vs. 47.3 hrs, P<0.001
- Mortality:
  - Overall: 20.3% vs. 12.7%, P<0.021
  - Gram negative: 25.3% vs. 8.3%, P<0.003

Angela M. Huang, and others. CID. 2013:57
### Influence of Rapid Bacterial Identification by Molecular Methods Combined with Antimicrobial Stewardship

<table>
<thead>
<tr>
<th>Test</th>
<th>Antibiotic Use</th>
<th>Outcomes</th>
<th>Cost Savings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cepheid Xpert&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6 days sooner for MSSA</td>
<td>LOS shorter by 6.2 days</td>
<td>$21,000</td>
</tr>
<tr>
<td>PNA FISH – CoNS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>More patients received 1 vanco dose (43% vs. 15%; p&lt;0.005)</td>
<td>LOS shorter by 2 days</td>
<td>$4,000</td>
</tr>
<tr>
<td>PNA FISH – Enterococcus sp&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Time to appropriate antibiotics 1.8 days sooner for VRE</td>
<td>Decreased mortality (26% vs. 45%, p=0.04)</td>
<td>N/A</td>
</tr>
<tr>
<td>Verigene BC-GP&lt;sup&gt;d&lt;/sup&gt; (Enterococcal bacteremia)</td>
<td>Time to appropriate antibiotic 23.4 hrs faster; for VRE 31.1 hrs faster</td>
<td>LOS: 43.5 days vs. 22.2, P=0.14 Attributed mortality: 2.1% vs. 14.2%, P=0.07</td>
<td>$60,729</td>
</tr>
</tbody>
</table>

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b. GN Forrest. JAC. (2006). 58; 154-158
Molecular Multiplex Panels for Identification of Positive Blood Cultures – Verigene®

**Gram Positives (BC GP)**
- *Staphylococcus* sp
  - *mecA* gene for *S. aureus* and *S. epidermidis*
- *Enterococcus faecalis* and *faecium*
  - Van A
  - Van B
- *Streptococcus* sp
  - *S. pyogenes*
  - *S. agalactiae*
  - *S. pneumoniae*
- *Listeria monocytogenes*

**Gram Negatives (BC GN)**
- *Enterobacteriaceae*
  - *E. coli*
  - *Enterobacter* sp.
  - *K. pneumoniae/oxytoca*
  - *Proteus* sp.
  - *Citrobacter* sp.
- *Pseudomonas aeruginosa*
- *Acinetobacter* sp.

**RESISTANCE MARKERS:**
- CTX-M (1 of the 3 ESBLs)
- Carbapenemases (KPC, NDM, VIM, IMP, OXA)
Turnaround time for workup of *Staphylococcus aureus*

Kruskal Wallis with Dunn’s correction

MMP=mutiplex molecular panel
TC=tube coagulase
PNAF=peptide nucleic acid fluorescent in situ hybridization
Influence of Timing of Identification on Antibiotic Use: MSSA

<table>
<thead>
<tr>
<th></th>
<th>MMP N=50</th>
<th>PNAF N=50</th>
<th>TC N=50</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Received vancomycin</td>
<td>46 (92%)</td>
<td>46 (92%)</td>
<td>44 (88%)</td>
<td>NS</td>
</tr>
<tr>
<td>Median [95% CI] number</td>
<td>2 [1-3]</td>
<td>5 [4-5]</td>
<td>4 [3-4]</td>
<td>0.001</td>
</tr>
<tr>
<td>vancomycin doses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (%) of patients who</td>
<td>21/45 (47%)</td>
<td>4/45 (9%)</td>
<td>4/44 (9%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>received a single</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vancomycin dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nafcillin or cefazolin, N (%)</td>
<td>44 (88%)</td>
<td>40 (80%)</td>
<td>37 (74%)</td>
<td>0.2</td>
</tr>
<tr>
<td>Time (hours) to Nafcillin</td>
<td>27</td>
<td>53</td>
<td>62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>or Cefazolin</td>
<td>[25-34]</td>
<td>[42-64]</td>
<td>[51-74]</td>
<td></td>
</tr>
</tbody>
</table>

*Kruskal Wallis with Dunn’s correction for continuous variables; Chi Square for proportions
CI=confidence interval

MMP=multiplex molecular panel
TC=tube coagulase
PNAF=peptide nucleic acid fluorescent in situ hybridization

Unpublished
## Length of Stay and Mortality

<table>
<thead>
<tr>
<th></th>
<th>MMP (N=65)</th>
<th>Control (N=130)</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hospital LOS, days</strong></td>
<td>6 [6-9]</td>
<td>8 [7-9]</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>MSSA LOS</strong></td>
<td>6 [5-7]</td>
<td>7 [6-9]</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>MRSA LOS</strong></td>
<td>12 [6-26]</td>
<td>9 [7-13]</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>N with ICU stay</strong></td>
<td>33 (51%)</td>
<td>74 (57%)</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>ICU LOS, days</strong></td>
<td>5 [4-7]</td>
<td>6 [5-7]</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>MSSA LOS</strong></td>
<td>4 [3-6]</td>
<td>6 [4-7]</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>MRSA LOS</strong></td>
<td>7 [5-23]</td>
<td>6 [4-8]</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Mortality, N (%)</strong></td>
<td>4 (6.2%)</td>
<td>8 (6.2%)</td>
<td>ns</td>
</tr>
</tbody>
</table>

1. Both control groups combined for analysis since no differences in antibiotic use.
2. Wilcoxon Rank Sum for continuous variables; Fisher’s exact test for proportions

MMP = multiplex molecular panel
Total Hospital Cost: MSSA

- Total Hospital Cost, Median [95% CI]
  - MMP (N=50): $16,112 [$13,254-$23,071]
  - Control (N=150): $21,300 [$18,282-$26,487]

MMP=multiplex, molecular panel
CI=Confidence Interval
Groups compared with Wilcoxon Rank Sum
Unpublished
Current Rapid Strategy

POSITIVE BLOOD CULTURE

GRAM STAIN

MALDI

1. Multiplex Molecular Panel
2. Subculture

Streptococcus spp
1. Report presumptive Streptococcus
2. Subculture

S. aureus
E. faecium/faecalis
E. coli/K. pneumoniae

1. Report Coag Neg Staphylococcus
2. Subculture

Staphylcocccus sp-not aureus
1. Report Coag Neg Staphylococcus
2. Subculture
How to Interpret Reports

• Methicillin resistance detected=MRSA
• Vancomycin resistance detected=VRE

• Gram negative resistance much more complicated!
  – GNR can be resistant in the absence of resistance genes
  – We won’t report NEGATIVE resistant genes except for
  – Carbapenem resistance not detected only used for *E coli* and *Klebsiella* spp.
Case 1

• 28 y/o healthy woman was admitted with abrupt onset of fever (103), rigors, headache, nausea, vomiting, then diarrhea (watery, no blood or mucous) and abdominal pain. One day earlier she attended a wedding reception at a local restaurant.

• No respiratory complaints

• Temp 40.1, HR 130, BP 107/53, RR 22

• Uncomfortable, diffuse abdominal tenderness with no peritoneal signs
Case 1

- WBC 10.8 with 18% bands,
- Chemistry panel and LFTs within normal limits
- CT Abdomen – diffuse colitis

The problem is:
A. Above the diaphragm
B. Below the diaphragm
C. Both
CASE 1

• What is the most likely cause of infection?

1) Non-typhoidal Salmonella
2) Shigella spp
3) E. coli O157:H7
4) Norovirus
5) Campylobacter jejuni
6) Rotavirus
Case 1


2. Stool cultures – negative

1. Viral molecular panel – negative for respiratory viruses
The Age of PCR for Everything?
Burden of Gastrointestinal Illness

179 Million cases of acute diarrhea in the U.S. each year

$6 Billion/year

Foodborne-associated infections in the U.S. = 48 Million

Rates of Bacterial Pathogens per 100,000 People, 2012

Shigella = 2.3 cases/100,000 people in 2011

Fecal Leukocytes or Lactoferrin Can Help

NON-INFLAMMATORY
- Vibrio cholera
- E. Coli (EPEC)
- Clostridium perfringens
- S. aureus, B. cereus
- Giardia
- Norovirus
- Cryptosporidium
- Rotavirus

INFLAMMATORY
- Vibrio parahemolyticus
- Shigella
- Salmonella
- Campylobacter jejuni
- E. Coli (EIEC, EHEC)
- Clostridium difficile
- E. histolytica
History Matters

- Presence of fever, tenesmus, blood
- Abdominal pain
- Seafood and other food exposure
- Antibiotic use
- Weight loss
- Travel
- Outbreaks
- Sexual exposures
- Immunosuppression
Indications for Stool Testing

- Dehydration
- Passage of small volume stool with blood and mucus
- Bloody diarrhea
- Acute diarrhea that is severe or associated with fever
- Persistent diarrhea (>14 days)
- Diarrhea associated with severe abdominal pain
- Recent use of antibiotics
- Elderly or immunocompromised
- Persons employed as food handlers or daycare workers
Diagnostic Tests Available in the Central Lab for Targeted Testing of Gastroenteritis

- **Stool Culture**
  - Salmonella
  - Shigella
  - Campylobacter
  - *Plesiomonas shigaloides*
  - Yersinia
  - Vibrio
  - Aeromonas

- **Norovirus PCR**
  - Cepheid Xpert (NEW!)

- **Rotavirus Antigen Detection**

- **Clostridium difficile**
  - GDH/Toxin A and B EIA
  - *C diff* PCR

- **E. coli O157/H7 (culture)**
- **Shiga Toxin** detection
- **Giardia/Cryptosporidium Antigen Testing**

- **Microscopy for Ova and Parasites**
  - ONLY WITH TRAVEL HISTORY
FilmArray™ GI Panel (GIPCR) $$$$

- **Bacteria**
  - *Campylobacter*
  - *Clostridium difficile*
  - *Plesiomonas shigelloides*
  - *Salmonella*
  - *Y. enterocolitica*
  - *Vibrio (parahaemolyticus, vulnificus and cholerae)*
  - *EAEC/EPEC/ETEC/STEC*
  - *Shigella/Enteroinvasive E. coli*

- **Parasites**
  - *Cryptosporidium*
  - *Cyclospora cayetanensis*
  - *Entamoeba histolytica*
  - *Giardia lamblia*

- **Viruses**
  - *Adenovirus F 40/41*
  - *Astrovirus*
  - *Norovirus GI/GII Rotavirus A*
  - *Sapovirus (I, II, IV and V)*
Verigene® Enteric Pathogens Test (EPPCR) $\$$

- **PCR with microarray based method**
- **Bacteria**
  - *Campylobacter* group
  - *Salmonella* spp.
  - *Shigella* spp.
  - *Vibrio* Group
  - *Yersinia enterocolitica*
- **Toxins**
  - Shiga Toxin 1 (stx1)
  - Shiga Toxin 2 (stx2)
- **Viruses**
  - Norovirus
  - Rotavirus
Organisms Detected with GIPCR

• **Objective:** Test throughput of new configuration

• **Method:** Tested convenience sample of randomly selected stool samples
  – Results were not communicated to clinicians

• **Study Period:** 1/2015-3/2015

• 464 stool samples processed

• 193 (43%) positive for any target
Tests Ordered by Clinicians and Number of Positive Tests

- **Total ordered**
- **Positive by ordered test**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total Ordered</th>
<th>Positive by Ordered Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella</td>
<td>401</td>
<td>10</td>
</tr>
<tr>
<td>Camplyobacter</td>
<td>401</td>
<td>6</td>
</tr>
<tr>
<td>Shigella</td>
<td>401</td>
<td>1</td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>374</td>
<td>47</td>
</tr>
<tr>
<td>Giardia</td>
<td>256</td>
<td>3</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>68</td>
<td>4</td>
</tr>
<tr>
<td>O&amp;P</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>EHEC</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>Norovirus</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Yersinia</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

Unpublished
Frequency of Pathogens Detected in Stool Samples by Diagnostic Approach

By FilmArray GI Panel

- Clostridium difficile: 56
- Norovirus: 44
- Rotavirus: 2
- EAEC: 25
- EPEC: 25
- Campylobacter: 25
- Salmonella: 14
- ETEC: 11
- Astrovirus: 10
- Shigella/EIEC: 10
- Sapovirus: 9
- Giardia lamblia: 9
- Adenovirus F40/41: 4
- Cryptosporidium: 5
- Plesiomonas shigelloides: 3
- Yersinia enterocolitica: 3
- STEC: 1

By ordered test

Unpublished
Numbers of Tests Positive for Multiple Pathogens

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Frequency in samples with multiple pathogens</th>
<th>Total frequency by FilmArray GI Panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostridium difficile (toxin)</td>
<td>16</td>
<td>56</td>
</tr>
<tr>
<td>Norovirus (GI/GII)</td>
<td>9</td>
<td>44</td>
</tr>
<tr>
<td>EAEC</td>
<td>16</td>
<td>25</td>
</tr>
<tr>
<td>EPEC</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>Rotavirus A</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Campylobacterspecies</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Salmonella</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>ETEC</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Astrovirus</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Shigella/EIEC</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Giardia lamblia</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Adenovirus F 40/41</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Plesiomonas shigelloides</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Versinia enterocolítica</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>STEC</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
Recommendations for use of molecular GI panels

• **Targeted Testing Based on History preferred** – If >2 tests are needed, consider multiplex panel

• **Acute Diarrhea** – Verigene EP (CODE=EPPCR)
  – Diarrhea severe enough to require hospital admission
  – Other tests as indicated (C. diff, Giardia/Crypto antigen)
  – Discourage use for diarrhea that develops >72 hours after admission

• **Complex Diarrhea** – BioFire GIP (CODE=GIPCR)
  – Diarrhea severe enough to require hospital admission
  – Returned travellers
  – Immunosuppressed
    • Solid organ transplant
    • Hematologic malignancy
  – Discourage use for diarrhea that develops >72 hours after admission
What are the financial implications of multiplex PCR testing if 5% of patients tested with multiplex panels in US?

- NO OUTCOMES DATA YET!

- EPPCR (Verigene) average retail cost $80/test and estimated charge $450 (based on estimates from other labs)
  - $716,000,000 acquisition cost to labs
  - $4,027,500,000 estimated charge to patients

- GIPCR (BioFire) average retail cost $120/test and charge to patient $750 (estimate from other lab)
  - $1,074,000,000 acquisition cost to labs
  - $$6,712,500,000 in charges

- May be more cost effective than multiple targeted tests depending on what is ordered
Diagnostic test available at Intermountain Central Lab

- Algorithm using QuickCheck combined EIA with reflex to PCR for toxin B
  1. Glutamate dehydrogenase (GDH) antigen
  2. Toxin A/B antigen
- Results:
  - GDH positive:toxin positive= POSITIVE
  - GDH negative:toxin negative= NEGATIVE
  - Discordant GDH:toxin= INDETERMINATE
  • Negative PCR= NEGATIVE
  • Positive PCR= POSITIVE
Are Molecular Tests Overdiagnosing *C. difficile*?

1. What is the natural history of PCR positive patients with negative toxin immunoassay results?

2. How do outcomes compare to toxin+/PCR+ and toxin-/PCR- patients?

3. Do PCR positive patients with negative toxin results require treatment for CDI?

Christopher R. Polage, and others. JAMA Intern Med. Sept 8 2015
# Molecular testing of *C. difficile*

<table>
<thead>
<tr>
<th></th>
<th>Toxin+/PCR+</th>
<th>Toxin-/PCR+</th>
<th>Toxin-/PCR-</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>131 (9.3%)</td>
<td>162 (11.4%)</td>
<td>1123 (79.3%)</td>
</tr>
<tr>
<td><strong>Stool Count on Day 1, median (IQR)</strong></td>
<td>5 (3-6)</td>
<td>3 (2-5)</td>
<td>3 (2-5)</td>
</tr>
<tr>
<td><strong>Diarrhea, 15-day f/u</strong></td>
<td>16.7%</td>
<td>8.6%</td>
<td>6.7%</td>
</tr>
<tr>
<td><strong>Other diarrheal or GI Inflammatory process</strong></td>
<td>8 (6.1%)</td>
<td>27 (16.7%)</td>
<td>161 (14.3%)</td>
</tr>
<tr>
<td><strong>C. difficile Toxin B, median (IQR), ng/mL</strong></td>
<td>640.8 (172.5-1194.0)</td>
<td>1.1 (0.3-2.5)</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Received Treatment</strong></td>
<td>100%</td>
<td>40.7%</td>
<td>3 (0.3%)</td>
</tr>
<tr>
<td><strong>CDI related complications</strong></td>
<td>10 (7.6%)</td>
<td>0</td>
<td>3 (0.3%)</td>
</tr>
<tr>
<td><strong>CDI related deaths</strong></td>
<td>11 (8.4%)</td>
<td>1 (0.6%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Adapted from Christopher R. Polage, and others. JAMA Intern Med. Sept 8 2015
When to Test for *C. difficile* Infection

**C**
- Container
  - Only test stool that conforms to its container

**D**
- Diarrhea
  - Only test after the patient has had at least 3 liquid stools within 24 hours

**I**
- Iatrogenic Causes Excluded
  - Hold off testing for 24-48 hours after laxative use or recent tube feed initiation, if patient is clinically stable
Pitfalls of *C. difficile* Testing

**Under Testing:**
- Missed cases lead to increased morbidity and mortality
- Increased nosocomial spread of *C. difficile*

**Over Testing:**
- Inflated rates of institutional *C. difficile*
- Unnecessary treatment with additional medication side effects, healthcare costs, disruption of GI microbiome and increased risk of recurrence

**Just right testing is the goal!**
What we hope to not see anymore!

12. Diarrhea, C. diff negative. We scheduled stool softeners during this admission. The patient's diarrhea has improved with most recent stool September 16, 2015.
Remember, ordering a diagnostic test is like picking your nose in public: you must first consider what you will do if you find something

-Catherine D. DeAngelis, MD