New Technologies for Detection of Enteric Pathogens

ALGORITHMS, APPLICATIONS, AND ADVANTAGES OF MOLECULAR DIAGNOSTICS

Blake W. Buchan, Ph.D., D(ABMM)
Assistant Professor of Pathology
Medical College of Wisconsin and Associate Director, Microbiology
Dynacare Laboratories
Milwaukee, WI
Disclosure

- Received travel support or honoraria from...
  - Nanosphere Inc.
  - Becton Dickinson and Company (BD)
  - Siemens
  - bioMerieux
  - GenMark
  - Quidel Corp
Tertiary care teaching hospital (600 beds)
- Dynacare Laboratory
- Service Froedtert Hospital, Two associated community Hospitals, Clinics, LTACs.
- 6-7k stool cultures/yr
- 9-10k C. difficile tests/yr
Experience with Enteric Pathogen NAATs

- **Research/Clinical trials**
  - BD MAX C. difficile
  - Portrait C. difficile
  - Verigene C. difficile
  - Ilumigene C. difficile
  - Lyra C. difficile
  - BD MAX EBP
  - ProGastro SSCS
  - Verigene ENT

- **Clinical use**
  - Xpert C. difficile
Diarrhea

- **Scope of problem**
  - Enteric illness affects millions yearly in US alone.
  - Second to respiratory illness for prevalence and reason for physician visit
  - Mortality in infants and elderly

- **Definition**
  - ≥ 3 unformed stools in 24 hr period

- **Causes**
  - **Foodborne**
    - Salmonella, Campylobacter, Y. enterocolitica, V. parahaemolyticus, ETEC, EPEC
  - **Environmental**
    - Cryptosporidium, Giardia, Entamoeba, Isospora/Cyclospora, Aeromonas, Plesiomonas
  - **Contagious**
    - Rotavirus, Norovirus, Shigella, V. cholerae, C. Difficile
  - **Toxin-mediated**
    - STEC, EHEC, C. perfringens, B. cereus, S. aureus
Diarrhea

- Characteristics/symptoms
  - Geography and travel history
  - Season
  - Age
  - Duration of symptoms
  - Watery? Bloody? Mucous?
  - Current outbreaks/epidemics
  - Community or hospital/LTC

Epidemiology?

<table>
<thead>
<tr>
<th>Agent</th>
<th>Cases/yr (K)</th>
<th>Hosp/yr (k)</th>
<th>% Hosp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norovirus</td>
<td>21,000</td>
<td>70</td>
<td>0.3%</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>1,200</td>
<td>23</td>
<td>1.9%</td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>2,400</td>
<td>40</td>
<td>1.6%</td>
</tr>
<tr>
<td><em>Shigella</em></td>
<td>500</td>
<td>5</td>
<td>1%</td>
</tr>
<tr>
<td><em>Vibrio</em></td>
<td>80</td>
<td>1</td>
<td>1.2%</td>
</tr>
<tr>
<td>STEC</td>
<td>250</td>
<td>3</td>
<td>1.2%</td>
</tr>
<tr>
<td><em>Yersinia</em></td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>1.0%</td>
</tr>
</tbody>
</table>

*V. cholerae < 20/yr, imported
C. difficile

- **Epidemiology**
  - Commensal in 2-5% of population (asymptomatic)
    - Alasmari et al CID → 21% colonization!!!
  - CDI following antibiotic course, nosocomial exposure to spores
    - More common in HAI and LTC due to these risk factors
  - Increasing prevalence in elderly from ~200 to >1,000 cs/100,000 population

Hurley et al., JAMA 2002
Rapid tests for *C. difficile*

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity (%) (95% CI)</th>
<th>Specificity (%) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premier toxin A+B</td>
<td>91.7 (84.7–96.1)</td>
<td>97.1 (95.1–98.4)</td>
</tr>
<tr>
<td>GA <em>Clostridium difficile</em> antigen</td>
<td>76.8 (67.7–84.4)</td>
<td>90.9 (88.0–93.3)</td>
</tr>
<tr>
<td>Ridascreen toxin A/B</td>
<td>66.7 (56.9–75.4)</td>
<td>95.1 (92.6–96.7)</td>
</tr>
<tr>
<td>Techlab toxin A/B II</td>
<td>90.7 (83.6–95.5)</td>
<td>95.7 (93.4–97.3)</td>
</tr>
<tr>
<td>Remel ProSpecT</td>
<td>89.8 (82.5–94.8)</td>
<td>92.6 (89.8–94.7)</td>
</tr>
<tr>
<td>Vidas <em>C. difficile</em> toxin A/B</td>
<td>89.8 (82.5–94.8)</td>
<td>96.7 (94.6–98.0)</td>
</tr>
<tr>
<td>Remel Xpect</td>
<td>77.8 (68.8–85.2)</td>
<td>98.8 (97.2–99.5)</td>
</tr>
<tr>
<td>Techlab Tox A/B Quik Chek</td>
<td>84.3 (76.0–90.6)</td>
<td>98.6 (96.9–99.4)</td>
</tr>
<tr>
<td>Premier Immunocard A + B</td>
<td>77.8 (68.8–85.2)</td>
<td>92.8 (90.1–94.9)</td>
</tr>
<tr>
<td>Techlab C. diff Chek-60</td>
<td>90.1 (81.6–95.1)</td>
<td>92.9 (90.1–95.0)</td>
</tr>
<tr>
<td>BD GeneOhm <em>C. difficile</em></td>
<td>92.2 (84.1–96.6)</td>
<td>94.0 (91.3–95.9)</td>
</tr>
</tbody>
</table>

$n \sim 500$ stools

Eastwood et al., JCM 2009
## Molecular assays

**TABLE 2** Comparison of four molecular tests to TBC/CCNA for detection of toxigenic *C. difficile*<sup>a</sup>

<table>
<thead>
<tr>
<th>Test</th>
<th>No. of specimens at site</th>
<th>No. of specimens with result</th>
<th>% sensitivity (CI)</th>
<th>% specificity (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portrait</td>
<td>540</td>
<td>109 31 398 2</td>
<td>98.2 (93–99)</td>
<td>92.8 (89–95)</td>
</tr>
<tr>
<td>Gene Xpert</td>
<td>275</td>
<td>58 18 199 0</td>
<td>100 (93–100)</td>
<td>91.7 (87–95)</td>
</tr>
<tr>
<td>GeneOhm</td>
<td>169</td>
<td>37 2 129 1</td>
<td>97.4 (86–99)</td>
<td>98.5 (94–99)</td>
</tr>
<tr>
<td>Illumigene</td>
<td>96</td>
<td>14 4 77 1</td>
<td>93.3 (68–99)</td>
<td>95.1 (87–98)</td>
</tr>
</tbody>
</table>

<sup>a</sup> TP, true positive; FP, false positive; TN, true negative; FN, false negative; CI, 95% confidence interval.

14/18 Xpert FP were positive by alternative NAAT

- Specificity for DNA 96.6%

Buchan et. al, JCM 2012
Clinical impact?

- High sensitivity/NPV → “I believe a negative result!!!”
  - Antimicrobial stewardship
    - Reduce duration of therapy/doses → hold empiric therapy & discontinue if negative result
  - Reduce cost/labor
    - Reduce repeat test volume, reduce time in isolation
Can we do this with community acquired illness?

- **Obstacles**
  - **Multitude of pathogens**
    - **Bacterial** → Salmonella, Campylobacter, Y. enterocolitica, V. parahaemolyticus, ETEC, EPEC, STEC, EHEC, B. cereus, S. aureus, C. perfringins, Shigella, V. cholera, Aeromonas spp. Plesiomonas spp.
    - **Viral** → Rotavirus, Norovirus, Adenovirus, Sapovirus
    - **Protozoan** → Cryptosporidium, Giardia, Isospora/Cyclospora, Entomobabia, Balantidium

*Rational set of targets based on...*

Community prevalence
- *Campylobacter, Salmonella*

Infection control
- *Shigella, EHEC*
Causes of community acquired illness

**CDC FoodNet 2011 Annual Report**

- **Salmonella**: 42%
- **Campylobacter**: 36%
- **Vibrio**: 1%
- **Cyclospora**: 0%
- **Cryptosporidium**: 7%
- **STEC (non 0157)**: 3%
- **STEC (0157)**: 2%
- **Yersinia**: 1%
- **Shigella**: 8%

*Does not include viral etiologies

**C-EnterNet 2009 Annual Report**

- **Campylobacteriosis**: 30%
- **Salmonellosis**: 30%
- **Giardiasis**: 18%
- **Listeriosis**: 1%
- **Cyclosporiasis**: 5%
- **Cryptosporidiosis**: 5%
- **Hepatitis A Virus infection**: 2%
- **Shigellosis**: 2%
- **Yersiniosis**: 2%
- **Amoebiasis**: 7%

*Does not include calicivirus
Causes of community acquired illness

Bacterial Enteric Pathogens Identified by WSLH from Samples Submitted by Wisconsin Clinical Laboratories, 2007
Are we really looking for *everything*?

- What does your lab report say for “negative” specimens?

- Is MAC sufficient for *Yersinia* spp?

- Is sweep ox sufficient for *Vibrio* spp?
Laboratory Diagnoses - Culture

- **Screen (Non-specific)**
  - Selective/differential media
    - BAP, MAC, XLD, HE, Campy, SMAC
  - Enrich
    - 42°C incubation, MAC broth
  - Additional media/biochems as needed
    - CIN, TCBS, “Sweep oxidase”

- **Confirm**
  - “Presumptive” colonies
    - API, Phoenix, Vitek2, RapID NF, MALDI-TOF (?)
  - “False positives”
    - Citro, Proteus, Pseudomonas, Serratia, VRE

- How are we doing?
  - TAT → 48-72h… clinically actionable? Infection control?
  - 95% of specimens “negative for x, y, z”
Choices and Algorithms

- **Enrichment broth**
  - GN – Salmonella/shigella
  - MAC – Initial enrichment for EHEC EIA
  - Campy CVA - Campylobacter
  → Add sensitivity but delay TAT and add cost

- **Plate ELISA/EIA**
  - Campylobacter, Stx1/2, norovirus
  → Can reduce TAT (direct testing), sensitivity +/-, add cost/labor

- **PCR**
  - Norovirus
  - Enteric Adenovirus
  - Rotavirus
  → LDTs, difficult for smaller labs to bring up.
  → Send-out, adds $$$/test and delay TAT
Enteric pathogen “panels”

Can a multiplex molecular test solve these problems?
- Sensitivity, speed, cost
Enteric pathogen “panels”

- **Potential benefits**
  - Higher sensitivity for detection/identification of enteric pathogens
  - More rapid TAT

- **Considerations**
  - Cost of molecular methods
  - Technical expertise
    - Test complexity
  - Level of automation
    - Sample to result? Off-line extraction or PCR?
  - Breadth of targets
    - All inclusive (viral, protozoan, bacterial, toxin)
    - Targeted (most common causes of CA-enteritis)
ProGastro SSCS

- **Approach** → “The big 4 (or 5)”
- **Targets** → *Salmonella, Shigella, Campylobacter, stx1, stx2*
- **FDA-cleared**
  - Cary Blair preserved stools
  - Stable at 2-8°C for up to 5 days, or frozen

Patient specimen in C&S/CaryBlair media → Dilute 50μl of sample into an additional 450μl of CaryBlair → Combine sample and Gastro Internal Control (GIC); transfer to easyMAG sample cartridge
ProGastro SSCS

- **Workflow - Batch**
  - Single extraction
  - 2 parallel PCR reaction “supermixes”

1. Perform extraction using Specific A 1.0.2 protocol
2. 5 µL of nucleic acid for SSC PCR reaction
3. 5 µL of nucleic acid for STEC PCR reaction
4. 85 minutes in SmartCycler
Performance

<table>
<thead>
<tr>
<th></th>
<th>TP</th>
<th>TN</th>
<th>FP</th>
<th>FN</th>
<th>Total</th>
<th>Sens</th>
<th>Spec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter*</td>
<td>20</td>
<td>1106</td>
<td>13(^a)</td>
<td>0</td>
<td>1139</td>
<td>100.0%</td>
<td>98.8%</td>
</tr>
<tr>
<td>Salmonella</td>
<td>20</td>
<td>1108</td>
<td>10(^b)</td>
<td>1</td>
<td>1139</td>
<td>95.2%</td>
<td>99.1%</td>
</tr>
<tr>
<td>Shigella</td>
<td>15</td>
<td>1118</td>
<td>6(^c)</td>
<td>0</td>
<td>1139</td>
<td>100.0%</td>
<td>99.5%</td>
</tr>
<tr>
<td>stx1/2 (EIA)</td>
<td>9</td>
<td>1121</td>
<td>9(^d)</td>
<td>0</td>
<td>1139</td>
<td>100.0%</td>
<td>99.2%</td>
</tr>
</tbody>
</table>

*\(C.\) coli or \(C.\) jejuni

\(^a\)6/13 positive by bi-directional sequencing
\(^b\)10/10 positive by bi-directional sequencing
\(^c\)6/6 positive by bi-directional sequencing
\(^d\)9/9 positive for stx1 or 2 by bi-directional sequencing
ProGastro SSCS

- Performance

### Post discordant resolution

<table>
<thead>
<tr>
<th></th>
<th>TP</th>
<th>TN</th>
<th>FP</th>
<th>FN</th>
<th>Total</th>
<th>Sens</th>
<th>Spec</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter</em></td>
<td>26</td>
<td>1106</td>
<td>7</td>
<td>0</td>
<td>1139</td>
<td>100.0%</td>
<td>99.4%</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>30</td>
<td>1109</td>
<td>0</td>
<td>0</td>
<td>1139</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td><em>Shigella</em></td>
<td>21</td>
<td>1118</td>
<td>0</td>
<td>0</td>
<td>1139</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>stx1/2</td>
<td>18</td>
<td>1121</td>
<td>0</td>
<td>0</td>
<td>1139</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

*C. coli or C. jejuni*
ProGastro SSCS

- Performance

<table>
<thead>
<tr>
<th></th>
<th>TP</th>
<th>TN</th>
<th>FP</th>
<th>FN</th>
<th>Total</th>
<th>Sens</th>
<th>Spec</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter</em></td>
<td>20</td>
<td>1113</td>
<td>0</td>
<td>6</td>
<td>1139</td>
<td>76.9%</td>
<td>100.0%</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>20</td>
<td>1108</td>
<td>1</td>
<td>10</td>
<td>1139</td>
<td>66.7%</td>
<td>99.9%</td>
</tr>
<tr>
<td><em>Shigella</em></td>
<td>15</td>
<td>1118</td>
<td>0</td>
<td>6</td>
<td>1139</td>
<td>71.4%</td>
<td>100.0%</td>
</tr>
<tr>
<td><em>stx1/2 (EIA)</em></td>
<td>9</td>
<td>1121</td>
<td>0</td>
<td>9</td>
<td>1139</td>
<td>50.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

*C. coli or C. jejuni

- Requires nucleic acid extraction and **two** different master mix reactions
  - Manual pipetting, setup

Buchan et al. JCM 2013
BD MAX EBP

- Approach “syndromic panels”
- FDA-cleared
  - EBP - *Salmonella*, *Shigella*, *Campylobacter*, *stx1*, *stx2*
- In development
  - Expanded bacterial – *Vibrio*, *Yersinia*, *Aeromonas*, ETEC, *Pliesiomonas*
  - Viral - Norovirus (genogroups I and II) and rotavirus, adenovirus, sapovirus, astrovirus
  - Parasite - *Giardia lamblia*, *Cryptosporidium*, *Entamoeba histolytica*
BD MAX EBP

- **Workflow – batch up to 24/run**
  - Cary Blair preserved or fresh stools
  - Stable at 2-8°C for up to 5 days

- Transfer 10 uL of preserved stool to Sample Buffer Tube
- Insert test strip/reagent packs
- Sample –result in ~2 hr.
### Performance

<table>
<thead>
<tr>
<th></th>
<th>BD MAX™ Enteric Bacterial Panel</th>
<th>Conventional culture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Campylobacter</strong>, prevalence = 5.3 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity (95 % CI)</td>
<td>100 (80.5–100)</td>
<td>52.9 (27.8–77)</td>
</tr>
<tr>
<td>Specificity (95 % CI)</td>
<td>100 (98.8–100)</td>
<td>100 (98.8–100)</td>
</tr>
<tr>
<td><strong>Shigella</strong>, prevalence = 4.4 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity (95 % CI)</td>
<td>100 (76.8–100)</td>
<td>71.4 (41.9–91.6)</td>
</tr>
<tr>
<td>Specificity (95 % CI)</td>
<td>100 (98.8–100)</td>
<td>100 (98.8–100)</td>
</tr>
<tr>
<td><strong>Salmonella</strong>, prevalence = 1.3 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity (95 % CI)</td>
<td>100 (39.8–100)</td>
<td>100 (39.8–100)</td>
</tr>
<tr>
<td>Specificity (95 % CI)</td>
<td>99.7 (98.2–100)</td>
<td>100 (98.8–100)</td>
</tr>
</tbody>
</table>
Performance
- Limit of detection
  - What factors impact culture sensitivity? Specificity?
    - Abx usage
    - Fastidious nature of bug
    - Screening medium?
    - Background flora?
BD MAX EBP

- **Performance**
  - Limit of detection
    - *Salmonella* burden decreases on progression
    - *Shigella* can be shed at low concentration
      - Asymptomatic carriage, still infection control concern
    - *Campylobacter* fastidious, difficult to isolate from flora
    - EHEC EIAs low sensitivity
      - LOD of EIA $\sim 10^6 - 10^7$ CFU/ml
      - Identify as little as 29% of positive specimens

Vallieres, E. et al. *JCM*. 2013
BD MAX EBP

**Salmonella spp.**

<table>
<thead>
<tr>
<th>Concentration (CFU/ml)</th>
<th>Strain 1</th>
<th>Strain 2</th>
<th>Strain 3</th>
<th>Strain 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^7</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>10^6</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>10^5</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>10^4</td>
<td>3/4</td>
<td>0/4</td>
<td>3/4</td>
<td>1/4</td>
</tr>
<tr>
<td>10^3</td>
<td>2/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>10^4</td>
<td>3/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>10^5</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>10^6</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>10^5</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>10^4</td>
<td>1/4</td>
<td>3/4</td>
<td>1/4</td>
<td>1/4</td>
</tr>
<tr>
<td>10^3</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
</tr>
</tbody>
</table>

Shigella spp.

BD MAX EBP

Anderson, N. et al. JCM. 2014
### TABLE 1 Comparative sensitivity of culture to the BD MAX EBP assay

<table>
<thead>
<tr>
<th>Isolate type (from prepared stool samples)</th>
<th>Sensitivity (%) by organism concentration and measurement method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^7 CFU/ml</td>
</tr>
<tr>
<td></td>
<td>BD MAX</td>
</tr>
<tr>
<td><em>Campylobacter</em>^{a}</td>
<td>NA^{b}</td>
</tr>
<tr>
<td>EHEC (O157)^{c}</td>
<td>100</td>
</tr>
<tr>
<td><em>Salmonella</em>^{d}</td>
<td>100</td>
</tr>
<tr>
<td><em>Shigella</em>^{d}</td>
<td>100</td>
</tr>
</tbody>
</table>

### Conclusions

- **Consistently more sensitive than culture**
  - 100% of positive cultures detected at concentration 10 to 100-fold below culture
  - Reliably (>90%) identify enteric pathogens to 10^4 CFU/ml

- **Clinical performance not affected by “background”**
  - Culture sensitivity dependent on quantity of flora
  - Culture specificity dependent on type of flora
  - Multiple NLFs or H2S (+) → multiple morphologies

*Anderson, N. et al. JCM. 2014*
Nanosphere Verigene EP

- Approach - Multiplex, microarray
- FDA-cleared
  - Bacteria – *Salmonella, Shigella, Campylobacter, Vibrio spp.*, *Y. enterocolotica*
  - Toxins – *stx1, stx2*
  - Viruses – *Norovirus, Rotavirus*

<table>
<thead>
<tr>
<th>Agent</th>
<th>Cases/yr (K)</th>
<th>Hosp/yr (k)</th>
<th>% Hosp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norovirus</td>
<td>21,000</td>
<td>70</td>
<td>0.3%</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>1,200</td>
<td>23</td>
<td>1.9%</td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>2,400</td>
<td>40</td>
<td>1.6%</td>
</tr>
<tr>
<td>Shigella</td>
<td>500</td>
<td>5</td>
<td>1%</td>
</tr>
<tr>
<td><em>Vibrio</em></td>
<td>80</td>
<td>1</td>
<td>1.2%</td>
</tr>
<tr>
<td>STEC</td>
<td>250</td>
<td>3</td>
<td>1.2%</td>
</tr>
<tr>
<td><em>Yersinia</em></td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>1.0%</td>
</tr>
</tbody>
</table>
Nanosphere Verigene EP

**Workflow – On demand**
- Cary Blair preserved or fresh stools
- Stable at 2-8°C for up to 5 days

- Transfer 50 uL of preserved stool to Cary Blair (dilution)
- Insert test reagents (extraction tray, utility tray, hybridization cartridge)
- Sample – result in <2.25 hr.
**Nanosphere Verigene EP**

- **Performance**
  - 354 stool specimens were evaluated
    - 196 prospective, 97 retrospective, and 55 simulated specimens

<table>
<thead>
<tr>
<th></th>
<th>TP</th>
<th>TN</th>
<th>FP</th>
<th>FN</th>
<th>Total</th>
<th>Sensitivity (CI)</th>
<th>Specificity (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter spp.</em></td>
<td>59</td>
<td>287</td>
<td>2</td>
<td>0</td>
<td>348</td>
<td>100.0% (92-100)</td>
<td>99.3% (97-100)</td>
</tr>
<tr>
<td><em>Vibrio spp.</em></td>
<td>9</td>
<td>338</td>
<td>0</td>
<td>1(^a)</td>
<td>348</td>
<td>90.0% (54-99)</td>
<td>100.0% (98-100)</td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td>35</td>
<td>310</td>
<td>1</td>
<td>2</td>
<td>348</td>
<td>94.6% (80-99)</td>
<td>99.7% (98-100)</td>
</tr>
<tr>
<td><em>Shigella spp.</em></td>
<td>3</td>
<td>345</td>
<td>0</td>
<td>0</td>
<td>348</td>
<td>100.0% (31-100)</td>
<td>100.0% (99-100)</td>
</tr>
<tr>
<td><em>Y. enterocolitica</em></td>
<td>4</td>
<td>344</td>
<td>0</td>
<td>0</td>
<td>348</td>
<td>100.0% (39-100)</td>
<td>100.0% (99-100)</td>
</tr>
<tr>
<td>stx1/2</td>
<td>21</td>
<td>324</td>
<td>2</td>
<td>1</td>
<td>348</td>
<td>95.5% (75-99)</td>
<td>99.4% (98-100)</td>
</tr>
<tr>
<td><em>Norovirus</em></td>
<td>14</td>
<td>334</td>
<td>0</td>
<td>0</td>
<td>348</td>
<td>100.0% (73-100)</td>
<td>100.0% (99-100)</td>
</tr>
<tr>
<td><em>Rotavirus</em></td>
<td>6</td>
<td>341</td>
<td>0</td>
<td>1</td>
<td>348</td>
<td>85.7% (42-99)</td>
<td>100.0% (98-100)</td>
</tr>
</tbody>
</table>

\(^a\)Simulated specimens spiked at 2x LoD
Luminex xTAG GPP

- **Approach - Multiplex, liquidarray**
- **FDA-cleared**
  - Bacteria – *Salmonella, Shigella, Campylobacter, E. coli 0157, ETEC (LS/ST), C. difficile*
  - Toxins – *stx1, stx2*
  - Viruses – *Norovirus, Rotavirus*
  - Protozoa - *Cryptosporidium, Giardia*
Luminex xTAG GPP

- **Workflow – Batch (up to 96*)**
  - FDA-cleared for *unpreserved stools only*
  - Extract stools → offline
  - PCR → offline (mastermix provided)
  - Hybridize
  - Analyze

- **45 min.**
- **1.5 h**
- **1.5 h**
- **10 min**

- Manual pipetting, PCR reaction setup, open transfer of amplicon
- Requires additional equipment → Extractor, PCR block
- 4 h TAT → Batch size limited by extraction step
Luminex xTAG GPP

- **Performance**
  - 901 Stools
  - xTAG GPP vs. SOC
    - Culture, EIA, DFA, Microscopy, PCR
  - Sensitivity ~95% overall
    - Range from 91%-100% for the various targets

Luminex xTAG GPP

- **Additional benefits?**
  - 30% total positivity
    - Up from 5% by bacterial culture
  - 9.5% of stools positive for multiple targets
    - *C. difficile* most common co-
      - Interpretation?
      - Cause vs. carriage?
  - Cdiff + Noro in LTC?
  - Cdiff + Campy in Community?

### Table 3. Targets implicated in co-infections.

<table>
<thead>
<tr>
<th>Target</th>
<th>No. of co-infections</th>
<th>% of co-infections</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium difficile</em> Toxin A/B^a</td>
<td>26</td>
<td>30.2</td>
</tr>
<tr>
<td>Norovirus</td>
<td>23</td>
<td>26.7</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>22</td>
<td>25.6</td>
</tr>
<tr>
<td>Shigella</td>
<td>21</td>
<td>24.4</td>
</tr>
<tr>
<td>Salmonella</td>
<td>18</td>
<td>20.9</td>
</tr>
<tr>
<td>Giardia</td>
<td>18</td>
<td>20.9</td>
</tr>
<tr>
<td>ETEC</td>
<td>13</td>
<td>15.1</td>
</tr>
<tr>
<td><em>E. coli</em> O157/STEC</td>
<td>11</td>
<td>12.8</td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td>7</td>
<td>8.1</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>7</td>
<td>8.1</td>
</tr>
<tr>
<td>Adenovirus 40/41</td>
<td>4</td>
<td>4.6</td>
</tr>
<tr>
<td>Rotavirus A</td>
<td>4</td>
<td>4.6</td>
</tr>
</tbody>
</table>

Luminex xTAG GPP

Additional benefits?

- Missed diagnosis?
  - 65% of positive results did not have appropriate test order

- C. diff...
  - 74% more diagnosis?
  - 74% more confusion?

Table 2. Unrequisitioned bacteria and parasites detected by xTAG GPP.

<table>
<thead>
<tr>
<th>Target</th>
<th>No. not requested by physician</th>
<th>% of total additional positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella</td>
<td>6</td>
<td>46</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Shigella</td>
<td>4</td>
<td>24</td>
</tr>
<tr>
<td>Clostridium difficile Toxin A/B</td>
<td>32</td>
<td>74</td>
</tr>
<tr>
<td>ETEC</td>
<td>12</td>
<td>63</td>
</tr>
<tr>
<td>E. coli O157</td>
<td>6</td>
<td>55</td>
</tr>
<tr>
<td>STEC</td>
<td>13</td>
<td>81</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Giardia</td>
<td>31</td>
<td>79</td>
</tr>
<tr>
<td>Entamoeba histolytica</td>
<td>10</td>
<td>71</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>Overall</td>
<td>137</td>
<td>65</td>
</tr>
</tbody>
</table>

FilmArray GI

- **Approach – Multiplex (Parallel singleplex)**
- **FDA-cleared**
  - *E. coli* - ETEC, EPEC, STEC/EHEC-O157:H7, EIEC, EAEC
  - Bacteria – *Aeromonas* spp., *Salmonella* spp., *Vibrio* spp., *V. cholerae*, *Shigella* spp., *S. dysenteriae*, *Campylobacter* spp., *Y. enterocolitica*, **C. difficile/Nap1**, *P. shigelloides*
  - Toxins – stx1, stx2
  - Viruses – *Norovirus (GI, GII, and GIV), Adenovirus F (40/41), Rotavirus (A, B, and C), Human Astrovirus, Sapovirus*
  - Parasites - *Cryptosporidium* group, *Giardia lamblia*, *Entamoeba histolytica*, *Cyclospora cayetanensis*
**FilmArray GI**

- **Workflow – On demand**
  - FDA-cleared for preserved and unpreserved stools
  - Dilute specimen into buffer
  - Inoculate test pouch
  - Sample to result in 1 hr

- Most comprehensive panel, easy to use, fast!
- Highest cost
- Throughput?
FilmArray GI

- **Performance**
  - 230 Prospective Stools, 270 Characterized stools
  - FilmArray GI vs. SOC
    - Culture, EIA, DFA, Microscopy, PCR
  - Sensitivity ~95% overall
    - Range from 91%-100% for the various targets
      - Norovirus 91.7%
      - C. difficile 91.7%
      - *Aeromonas* 23.8%
      - Shigella 90.9%
      - Adenovirus 90%

Khare. et al. *JCM* 2014
Additional benefits?

- 33% total positivity
  - 3% by bacterial culture

- Inclusive of Pathogenic *E. coli*
  - Undetectable by culture

- Noro + Sapo
  - Equal prevalence

- Cdiff accounts for 27% positives
  - Add’l diagnosis or confusion?

Khare. et al. *JCM* 2014
## Comparing platforms

<table>
<thead>
<tr>
<th></th>
<th>ProGastro</th>
<th>BD MAX</th>
<th>Verigene</th>
<th>xTAG GPP</th>
<th>FilmArray GI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Targets</strong></td>
<td>4</td>
<td>4</td>
<td>9</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td><strong>Automation</strong></td>
<td>Off-line Extraction, Manual PCR setup</td>
<td>Sample to Result</td>
<td>Sample to Result</td>
<td>Off-line Extraction, Manual PCR setup</td>
<td>Sample to result</td>
</tr>
<tr>
<td><strong>Technology</strong></td>
<td>RT-PCR</td>
<td>RT-PCR</td>
<td>PCR+Array</td>
<td>PCR+xTAG</td>
<td>Nested PCR</td>
</tr>
<tr>
<td><strong>Throughput</strong></td>
<td>Batch, limited by SmartCycler capacity</td>
<td>Batch, up to 24</td>
<td>1 sample/run</td>
<td>Batch, limited by Extractor capacity</td>
<td>1 sample/run</td>
</tr>
<tr>
<td><strong>TAT</strong></td>
<td>3 h</td>
<td>1.5 h</td>
<td>2 h</td>
<td>4 h</td>
<td>1 h</td>
</tr>
<tr>
<td><strong>Cost/test</strong></td>
<td>$$</td>
<td>$</td>
<td>$$$</td>
<td>$$$</td>
<td>$$$$$</td>
</tr>
</tbody>
</table>
What have we learned?

- Molecular panels for GI are...
  - More sensitive than routine culture/EIA methods
    - Additional 30%-150% positive results depending on target
    - Overall increase in positivity from ~5% to ~30% of stools
      - Detection of target unordered in up to 65% of positive results
  - Faster than routine methods
    - As little as 1h for S-R platforms, <24 h even if batched
  - Interpretation can be difficult for some targets
    - C. difficile in community patient with no abx, no healthcare exposure
  - Why test for CA-enteritis in inpatients? Why test giardia in winter?
    - Cost and reimbursement higher than routine methods
Cost/benefit – Can the lab/patients afford this?

- Prevalence vs. cost
  - *E. coli* containing *stx1* or *stx2*
  - Serotype *O157* associated with *stx2* carriage
    - HUS in 2-10% of infected peoples

**CDC recommendation (2009) and Joint Commission updated standard (2013) to culture for *O157* and use EIA/NAAT for *stx1/2***

<table>
<thead>
<tr>
<th>Organism(s)</th>
<th>% Prevalence&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. difficile</em></td>
<td>15.8</td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td>1.7</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>1.4</td>
</tr>
<tr>
<td><em>Aeromonas</em> spp.</td>
<td>0.9</td>
</tr>
<tr>
<td><em>Shigella</em> spp.</td>
<td>0.3</td>
</tr>
<tr>
<td><em>Vibrio</em> spp.</td>
<td>0.25</td>
</tr>
<tr>
<td>STEC</td>
<td>0.09</td>
</tr>
<tr>
<td><em>Plesiomonas shigelloides</em></td>
<td>0.08</td>
</tr>
</tbody>
</table>

<sup>a</sup> In a 13-month period for all except *C. difficile* (9.5 months).

Cost/benefit – Can the lab/patients afford this?

- Prevalence vs. cost
  - *E. coli* containing *stx1* or *stx2*
  - Serotype o157 associated with *stx2* carriage
    - HUS in 2-10% of infected peoples

*CDC recommendation (2009) and Joint Commission updated standard (2013) to culture for O157 and use EIA/NAAT for stx1/2*

**TABLE 3. Cost of stool testing, Upstate Medical University Hospital**

<table>
<thead>
<tr>
<th>Test</th>
<th>Cost per test ($)</th>
<th>Cost per positive test ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stool culture</td>
<td>11.88</td>
<td>255.42</td>
</tr>
<tr>
<td><em>C. difficile</em> PCR</td>
<td>52.80</td>
<td>333.77</td>
</tr>
<tr>
<td>Shiga toxin immunoassay</td>
<td>16.11</td>
<td>18,300.00</td>
</tr>
</tbody>
</table>

*Includes labor, reagents, and controls.*

Cost/benefit – *Can the lab/patients afford this?*

- Some simple math – Lab cost
  - **Culture**
  - Stool culture $10 + EIA $20 = $30
  - Multiply by 5% positivity = $600/positive stool
  - **Small panel**
  - $40 x 7% positivity = $571/positive stool
  - **Large panel**
  - $160 x 30% positivity = $533/positive stool

*Value!* We are “buying” positive results cheaper with molecular Dx!
Cost/benefit – Can the lab/patients afford this?

- Cost to the patient/healthcare insurer
  - **Culture CPT code(s)**
    - 87045 – Salmonella + Shigella: $12.97
    - 87046 – Add’l plates (Campy, SMAC): $9.69 ea.
    - 87427 – stx EIA: $16.49
    - 87077 – Workup of FP (API20): $16.63
  - **Total: $50.00-$65.00**

- **Molecular Dx CPT code(s)**
  - 87798 – Amplified probe, each organism: $48.80
  - ProGastro, BD MAX EBP x5 ➔ $244.00
  - Verigene ENT x9 ➔ $439.20
  - FilmArray GI x 22 ➔ $1073.60
  - Updated Molecular Dx code(s)…PENDING
    - 87631 – multiplex 3 - 5 targets ➔ $175.02
    - 87632 – multiplex 6 - 11 targets ➔ $291.18
    - 87633 – multiplex 6 - 11 targets ➔ $568.60
Is this the end of stool culture?

Probably not

- **Susceptibility testing**
  - Requires isolate

- **Epidemiology**
  - *Salmonella, Shigella, EHEC, Campylobacter* all reportable
  - Will your state lab/CDC accept preserved stool or broth?

- **Negative results**
  - All but largest panels lack sufficient coverage to rule out infectious process
  - “Only find what you are looking for”
Conclusion

- **Rapid rule out for common CA pathogens**
  - Positive stools may not require further work-up
    - Large panels $\rightarrow$ ID potential pathogen in up to 35% of stools
    - Small panels (ProGastro, MAX EBP) $\rightarrow$ ID potential pathogen in 5-7%
      - Negative result – focus further workup (Noro, TCBS, OX+, O&P)

- **Antibiotic stewardship**
  - Hold empiric therapy?
    - *Salmonella, EHEC, Noro* $\rightarrow$ No Abx
    - *Shigella*, protozoa $\rightarrow$ AST and treat

- **Infection control**
  - Identify outbreak or potential outbreak 48-72 h sooner! $\rightarrow$ Contain!!
    - Family members, school/daycare, LTC $\rightarrow$ Shigella, Noro, source EHEC