Advances in Laboratory Diagnosis of Gastroenteritis in Pediatrics

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I have no relevant disclosures.
Objectives:

At the conclusion of this lecture, the attendee will be able to:

1. Compare and contrast new and historical methods for laboratory diagnosis of pediatric gastroenteritis
2. Discuss the workup of pediatric specimens for gastroenteritis
3. Describe the impact of new methods on management of pediatric patients
Stool Sample
Acute Gastroenteritis (AGE):

Inflammation/infection of the digestive tract, particularly the stomach and intestines

(Sometimes referred to as the “stomach flu”)
Signs & Symptoms of AGE:

- Diarrhea and/or vomiting of >=7 days duration
- Fever
- Abdominal pain
- Anorexia
- Dehydration
Epidemiology:

- Worldwide children <5 yo
  - 3 – 5 billion cases
  - 2 million deaths

- US
  - 10% of hospital admissions
  - > 1.5 million OP visits
  - 300 deaths in < 5 yo
  - ~$1 billion
Gastrointestinal Pathogens

Bacterial (10 – 20%)
- *Salmonella*
- *Shigella*
- *Campylobacter*
- STEC
- *Yersinia enterocolitica*
- *Aeromonas* spp.
- *Plesiomonas* spp.
- *C. difficile* (?)

Viral (75 – 90%)
- Rotavirus
- Norovirus
- Enteric Adenoviruses
- Sapovirus
- Astrovirus

Parasitic (< 5%)
- *Giardia*
- *Cryptosporidium*
- *Cyclospora*
- *Isospora*
- *E. histolytica*
A word about *C. difficile*:

American Academy of Pediatrics!

“...ing in infants (younger than 12 months of age) is complicated by a high rate of asymptomatic colonization. Of these infants, should be limited to those with Hirschsprung disease or other severe mobility disorders or in an outbreak situation.”

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Presentation</th>
<th>Antimicrobial Tx</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>Sporadic</td>
<td>Special cases only:</td>
</tr>
<tr>
<td></td>
<td>Food-borne</td>
<td>&lt; 3 mos old</td>
</tr>
<tr>
<td></td>
<td>Reptiles</td>
<td>Chronic GI disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Immunocompromised</td>
</tr>
<tr>
<td><em>Shigella</em></td>
<td>Person-to-person</td>
<td>Special cases only:</td>
</tr>
<tr>
<td></td>
<td>Risk Factor: child care</td>
<td>Severe disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dysentery</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Immunocompromised</td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>Sporadic</td>
<td>Not routinely</td>
</tr>
<tr>
<td></td>
<td>Poultry</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unpasteurized milk</td>
<td></td>
</tr>
<tr>
<td><em>STEC</em></td>
<td>Outbreaks are common</td>
<td>No (Risk of HUS)</td>
</tr>
<tr>
<td></td>
<td>(petting zoos, drinking water)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Contamination of cattle feces</td>
<td></td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>Sporadic</td>
<td>Not Routinely</td>
</tr>
<tr>
<td></td>
<td>Swine-associated</td>
<td></td>
</tr>
</tbody>
</table>
Stool culture for bacterial gastroenteritis

- BAP
- XLD
- HE
- CAMPY
- SMAC
- Shiga broth
- CIN

API-20E

Microscan
Vitek
Phoenix

(MALDI-ToF)
Salmonella/Shigella

XLD

Lactose fermentor
(not Salmonella or Shigella)

H$_2$S positive Salmonella
Salmonella/Shigella

Lactose fermentor
(not *Salmonella* or *Shigella*)

Non-lactose fermentor
(possible *Salmonella* or *Shigella*)
Salmonella/Shigella

Serologic confirmation
Campylobacter spp.

- Gram negative curved rods
- growth at 42C, microaerophilic
- oxidase-positive
- hippurate
  - positive - *C. jejuni*
  - negative - *C. coli*
STEC:

SMAC

Serotyping for epidemiologic purposes
ELMO COLI'S HARD LESSON IN MARKETING...
Latest victim of a recent *E. coli* outbreak
Yersinia enterocolitica

- mannitol fermentation
- urease +

Dropping the concentration of cefsulodin to 4% will allow growth of Aeromonas
Viral (75 – 90%)
- Rotavirus
- Norovirus
- Enteric Adenoviruses
- Sapovirus
- Astrovirus
Parasitic (< 5%)

- Giardia
- Cryptosporidium
- Cyclospora
- Isospora
- E. histolytica
Giardia-Crypto EIA
Detects two most common agents of parasitic disease in the US
*Giardia lamblia*  
*Cryptosporidium parvum*
Sensitivity/specificity >90%
Rapid, technically easy
Ova & Parasite Exam

Direct examination - wet mounts
Concentration procedure
Permanent Smear - trichrome stain

Kinyoun (Cyclospora)
Trichrome stain (E. histolytica troph)
Trichrome stain (E. histolytica cyst)
Limitations of standard procedures

- Long TAT
- Labor intensive
- Insensitive
- Impacted by transport conditions
- Decreasing lack of expertise in specialty areas
Advanced Diagnostics – Molecular
FilmArray

**Simple:** Only 2 minutes of hands-on time  
**Easy:** No precise pipetting required  
**Fast:** Run time of about 1 hour
Verigene

- **Staph. aureus** oligonucleotide
- **Staph. epidermidis** oligonucleotide
- *Mediator oligonucleotide*
- *Staph. aureus-specific DNA*
- *Silver enhancement of gold nanoparticle*
- Gold nanoparticle with oligonucleotide probe
x-TAG

**Pre-PCR**
- Sample Pre-treatment: 45-60 minutes
- Nucleic Acid Extraction and Purification: 45 minutes
- Multiplex Application: 2.5 hours

**Post-PCR**
- Bead Hybridization and Detection: 1 hour
- Data Acquisition and Analysis by MAGPIX® or Luminex® 100/200™: 10 minutes
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>FilmArray GIP</th>
<th>xTAG GPP</th>
<th>Verigene EP</th>
<th>BD Max</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><em>Shigella</em></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>STEC (stx1/stx2)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td><em>Giardia</em></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cryptosporidium</em></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other parasites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cyclospora</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotavirus</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Norovirus</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Other viruses</td>
<td>AdV 40/41, AstroV, SapoV</td>
<td>AdV 40/41</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. difficile</em></td>
<td>A/B</td>
<td>A/B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td><em>Plesiomonas, EAEC, EPEC, ETEC</em></td>
<td>ETEC, <em>Vibrio cholerae</em></td>
<td><em>Vibrio spp.</em></td>
<td></td>
</tr>
</tbody>
</table>
Benefits of molecular procedures

• Multiplexed nature works well with syndromic testing
• Can provide a cost savings
• Exquisitely sensitive
• Much faster
What is the impact of these new technologies on patient management?
Multicenter Evaluation of the BioFire FilmArray Gastrointestinal Panel for Etiologic Diagnosis of Infectious Gastroenteritis

Sarah N. Buss, Amy Leber, Kimberle Chapin, Paul D. Fey, Matthew J. Bankowski, Matthew K. Jones, Margarita Rogatcheva, Kristen J. Kanack, Kevin M. Bourzac

Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska, USA; Department of Laboratory Medicine, Nationwide Children’s Hospital, Columbus, Ohio, USA; Departments of Pathology and Medicine, Lifespan Academic Medical Center, Providence, Rhode Island, USA; Diagnostic Laboratory Services, Inc., Aiea, Hawaii, USA; Departments of Pathology, Tropical Medicine, Medical Microbiology, and Pharmacology, John A. Burns School of Medicine and the University of Hawaii at Manoa, Honolulu, Hawaii, USA; BioFire Diagnostics, LLC, Salt Lake City, Utah, USA
Current Identification Methods

- Specimen receipt
- Culture setup
- Sendout referred
- Antigen Test Results Available
- Culture reading
- Bench side testing
- Setup automated ID panel
- Microscopic O & P performed
- O & P Results Available
- Organism Identification Available
- Sendout Results Available

Time to ID:
- ID using FA (~2 h)
- 0
- 8 h
- 24 h
- 30 h
- 48 h
- >1 wk
TABLE 2 Demographic characteristics of study specimens

<table>
<thead>
<tr>
<th>Patient subset</th>
<th>&lt;1</th>
<th>1–5</th>
<th>6–12</th>
<th>13–21</th>
<th>22–64</th>
<th>&gt;=65</th>
<th>Total no. (%) of specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Outpatient</td>
<td>70</td>
<td>48</td>
<td>216</td>
<td>187</td>
<td>102</td>
<td>83</td>
<td>41 72</td>
</tr>
<tr>
<td>Hospitalized</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>24 27</td>
</tr>
<tr>
<td>ER b</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>4  5</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>49</td>
<td>224</td>
<td>194</td>
<td>104</td>
<td>89</td>
<td>69 104</td>
</tr>
</tbody>
</table>

38% adult
62% pediatric
54% female
46% male

* M, male; F, female.
* ER, emergency room.

TABLE 3 Total number of FilmArray GI Panel-positive specimens by number of detections

<table>
<thead>
<tr>
<th>No. of potential pathogens in FilmArray GI Panel result</th>
<th>No. of specimens (n = 1,556)</th>
<th>% of total (% positives)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detected (at least one)</td>
<td>832</td>
<td>53.47 (100)</td>
</tr>
<tr>
<td>One</td>
<td>570</td>
<td>36.63 (68.51)</td>
</tr>
<tr>
<td>Two</td>
<td>199</td>
<td>12.79 (23.92)</td>
</tr>
<tr>
<td>Three</td>
<td>50</td>
<td>3.21 (6.01)</td>
</tr>
<tr>
<td>Four</td>
<td>9</td>
<td>0.58 (1.08)</td>
</tr>
<tr>
<td>Five</td>
<td>3</td>
<td>0.19 (0.36)</td>
</tr>
<tr>
<td>Six</td>
<td>1</td>
<td>0.06 (0.12)</td>
</tr>
</tbody>
</table>

31.49%
Co-Infxn
FilmArray performed with excellent sensitivity and specificity as compared to a variety of comparator assays.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>No. of detections&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Sensitivity/PPA&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Specificity/NPA&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. difficile</td>
<td>165/204</td>
<td>34/35 97.1 % 85.1–99.9</td>
<td>1,497/1,521 98.4 97.7–99.0</td>
</tr>
<tr>
<td>P. shigelloides</td>
<td>3/18</td>
<td>31/31 100 % 88.8–100</td>
<td>1,538/1,553 99.0 98.4–99.5</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>31/37</td>
<td>0/0</td>
<td>1,519/1,525 99.6 99.1–99.9</td>
</tr>
<tr>
<td>Vibrio spp.</td>
<td>0/2</td>
<td>0/0</td>
<td>1,554/1,556 99.9 99.5–100</td>
</tr>
<tr>
<td>Y. cholerae</td>
<td>0/1</td>
<td>1/1</td>
<td>1,555/1,556 99.9 99.6–100</td>
</tr>
<tr>
<td>Enterocolitica</td>
<td>1/1</td>
<td>1/1</td>
<td>1,555/1,556 99.9 99.8–100</td>
</tr>
<tr>
<td>EAEC</td>
<td>83/109</td>
<td>82/83 98.8 % 93.5–100</td>
<td>1,446/1,473 98.2 97.3–98.8</td>
</tr>
<tr>
<td>EPEC</td>
<td>317/348</td>
<td>314/317 99.1 % 97.3–99.8</td>
<td>1,167/1,201 97.2 96.1–98.0</td>
</tr>
<tr>
<td>ETEC</td>
<td>22/31</td>
<td>22/22 100 % 84.6–100</td>
<td>1,525/1,534 99.4 98.9–99.7</td>
</tr>
<tr>
<td>STEC</td>
<td>33/38</td>
<td>33/33 100 % 89.4–100</td>
<td>1,518/1,523 99.7 99.2–99.9</td>
</tr>
<tr>
<td>E. coli O157</td>
<td>3/4</td>
<td>3/3</td>
<td>34/35 97.1 % 85.1–99.9</td>
</tr>
<tr>
<td>Shigella spp./EIEC (culture)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>49 (31) 49</td>
<td>47/49 95.9 % 86.0–99.5</td>
<td>1,532/1,538 99.6 99.2–99.9</td>
</tr>
<tr>
<td>Cryptosporidium spp.</td>
<td>18/24</td>
<td>18/18 100 % 81.5–100</td>
<td>1,537/1,537 100 99.8–100</td>
</tr>
<tr>
<td>C. cayetanensis</td>
<td>19/19</td>
<td>19/19 100 % 82.4–100</td>
<td>1,556/1,556 100 99.8–100</td>
</tr>
<tr>
<td>E. histolytica</td>
<td>0/0</td>
<td>0/0</td>
<td>1,529/1,536 99.5 99.1–99.8</td>
</tr>
<tr>
<td>G. lamblia</td>
<td>20/27</td>
<td>20/20 100 % 83.2–100</td>
<td>1,499/1,512 99.1 98.5–99.5</td>
</tr>
<tr>
<td>Adenovirus F 40/41</td>
<td>44/55</td>
<td>42/44 95.5 % 84.5–99.4</td>
<td>1,548/1,549 99.9 99.6–100</td>
</tr>
<tr>
<td>Astrovirus</td>
<td>7/8</td>
<td>7/7</td>
<td>1,483/1,501 98.8 98.1–99.3</td>
</tr>
<tr>
<td>Norovirus GI/GII</td>
<td>55/70</td>
<td>52/55 94.5 % 84.9–98.9</td>
<td>1,538/1,550 99.2 98.7–99.6</td>
</tr>
<tr>
<td>Rotavirus A</td>
<td>6/18</td>
<td>6/6</td>
<td>1,497/1,510 99.1 98.5–99.5</td>
</tr>
<tr>
<td>Sapovirus</td>
<td>46/59</td>
<td>46/46 100 % 92.3–100</td>
<td>1,497/1,510 99.1 98.5–99.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of positive detections for the FilmArray (FA) and comparator (C) assays.

<sup>b</sup> Sensitivity and positive predictive accuracy (PPA) calculated as TP/(TP + FN) and 95% CI.

<sup>c</sup> NA: Not applicable.

<sup>d</sup> Shigella spp./EIEC (culture) results may vary depending on the specific strain and assay conditions.
Of the FN cases, 9/14 (64%) remained unresolved, while 5/14 (36%) showed that FA missed the target.

Of the FP cases, 33/237 (14%) remained unresolved, 5/237 (2%) showed that FA had some level of cross-reactivity, and 199/237 (84%) were confirmed using secondary testing.
Summary/Implications

• The FilmArray multiplex PCR assay provides a sensitive and specific syndromic approach to laboratory detection of microorganisms that cause IGE

• Laboratories must determine the performance of each target prior to patient care testing

• Laboratories must also determine their own approach to testing for:
  • Cdiff: infants <1 can be carriers; requires specimen restrictions for best performance
  • Aeromonas: depending on local prevalence may have to continue to offer Aeromonas culture
  • Public Health: still requires an isolate for follow-up testing (PFGE, serotyping, AST, etc.)
Summary

- Acute gastroenteritis is a major cause of morbidity and mortality in pediatrics
- Fast, reliable, sensitive, and specific methods are needed for laboratory diagnosis of AGE
- Multiplex PCR assays provide a sensitive and specific syndromic approach
- May not be able to discontinue culture completely, especially since PHL still require an isolate for follow-up/epidemiologic testing
The only comprehensive online resource for clinical microbiologists.

- **Q & A** is always ready to receive your microbiology questions and answers them in three days or less, providing science-based responses usually with relevant sources.
- **Cumitechs** references for bench microbiology and best practices for the clinical laboratory.
- **Hot Topics** offers you an expert’s presentation on a hot topic, current event, or controversial issue and their feedback to your questions.
- **Bench Work Resources** provides a wealth of information needed for the day-to-day work of diagnostic microbiologists and immunologists.
- **ASM Programs** can be found on one site that houses all of ASM's clinical content.
- **Lab Management** links users to a variety of tools that help facilitate and optimize the work of the laboratory manager.

http://clinmicro.asm.org
Questions?