Testing for Gastrointestinal Pathogens: How and Why a Community Hospital Chose the Molecular Approach

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Prior Speaker Honorarium: BD
Testing for Gastrointestinal Pathogens: How and Why a Community Hospital Chose the Molecular Approach

Overview

1. Case Discussions: where is the clinical value?
2. What is conventional stool culture missing?
3. Implications of molecular testing on workflow
4. Logistics of verification of a new gold standard
Case One

54F admitted at 09/29 from the community with a four day history of diarrhea
54F admitted 09/29 at 0228h

- Born in Canada, no foreign travel, family well
- Roux-en-Y in 1992 with subsequent reversal
- Esophageal strictures complicating gastric bypass in 2013; undergoes dilatation procedure monthly
- No recent antibiotics, PPIs, hospital stay
- Unwell x 4d with watery stool, abdominal cramps, nocturnal symptoms.
- Tender on palpation to the lower quadrants
- WBC 9.8
09/29 @2045h
Stool for *C. difficile* Cytotoxin PCR
POSITIVE
54F Case Evolution 09/30

- T 37.9 CT scan: diffuse colitis
- WBC 5.2
- *C. difficile* Ag POSITIVE, EIA toxin NEGATIVE

Isolate?  
Treat for CDI?  
Admit or Discharge?
• 1416 patients at a single centre tested and reports by MeridianTox A/B (Tox 1); PCR test results not reported
• Performed in background: PCR, CCNA, C. difficile load and toxin quantification, lactoferrin
• Outcomes: duration of diarrhea, CDI-related complications, death due to CDI
CDI Outcomes based on PCR/EIA Testing

Figure 2. Kaplan-Meier Curves of Time to Resolution of Diarrhea by Clostridium difficile Test Group

<table>
<thead>
<tr>
<th>No. at risk</th>
<th>Tox+/PCR+</th>
<th>Tox-/PCR+</th>
<th>Tox-/PCR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>131</td>
<td>62</td>
<td>41</td>
<td>29</td>
</tr>
<tr>
<td>162</td>
<td>60</td>
<td>29</td>
<td>21</td>
</tr>
<tr>
<td>1123</td>
<td>328</td>
<td>172</td>
<td>99</td>
</tr>
</tbody>
</table>

Duration of Diarrhea, Including Day of Testing, d

Proportion With Diarrhea
CDI Outcomes based on PCR / EIA / CCNA

- All CDI-related complications in the EIA+/PCR+ group (11/131); none in 161 EIA-/PCR+ group.
- Subgroup: 48/162 were EIA Tox-/ CCNA Tox+/PCR+
  - The subgroup had a lower toxin load, similar outcomes to (EIA) Tox-/PCR+
  - One EIA Tox-/PCR+ case subsequently developed recurrent CDI; care withdrawn due to underlying illness and death within 30 days
Better outcomes in first group: are they colonized

Such patients less likely to have a WBC > 15
Better outcomes in first group: are they colonized

Such patients less likely to have a WBC > 15
Two Step Testing for *C. difficile*

12420 fecal samples tested by toxigenic culture and CTA

- All tested in parallel by MeridianTox A/B (Tox 1), Techlab Tox A/B (Tox 2), Techlab GDH, GeneXpert PCR (NAAT)

- WBC, Cr, Albumin within three days of fecal sample
Two Step Testing for *C. difficile*

Outcomes

<table>
<thead>
<tr>
<th>CTA positive</th>
<th>NAAT positive/ CTA negative</th>
<th>CTA and NAAT negative</th>
<th>CTA positive vs NAAT positive/CTA negative p value</th>
<th>CTA positive vs CTA and NAAT negative p value</th>
<th>NAAT positive/CTA negative vs CTA and NAAT negative p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>CTA positive</td>
<td>NAAT positive/</td>
<td>CTA positive vs NAAT positive vs CTA negative</td>
<td>CTA positive vs CTA and NAAT NAAT negative</td>
<td>CTA positive vs CTA and NAAT negative vs CTA</td>
</tr>
<tr>
<td>Female (%)</td>
<td>435</td>
<td>311</td>
<td>3943</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mean age (years; SD)</td>
<td>69 (20)</td>
<td>64 (22)</td>
<td>64 (21)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mean white cell count (x10^9/L; SD)</td>
<td>12.4 (8.9)</td>
<td>9.9 (6.6)</td>
<td>10.012.0</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean rise in creatinine (%; SD)</td>
<td>37% (63)</td>
<td>49% (132)</td>
<td>34% (81)</td>
<td>0.0222</td>
<td>0.3018</td>
</tr>
<tr>
<td>&gt; 100% rise in creatinine (%)</td>
<td>40/316 (13%)</td>
<td>30/245 (12%)</td>
<td>32/3163 (9%)</td>
<td>0.0085</td>
<td>0.0085</td>
</tr>
<tr>
<td>Mean albumin (g/L; SD)</td>
<td>31 (7)</td>
<td>33 (8)</td>
<td>33 (8)</td>
<td>0.9328</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Albumin &lt; 20 g/L (%)</td>
<td>13/344 (4%)</td>
<td>15/258 (6%)</td>
<td>16/3232 (5%)</td>
<td>&lt;0.0001</td>
<td>0.0456</td>
</tr>
<tr>
<td>Died (%)</td>
<td>72/435 (16-5%)</td>
<td>30/311 (9.7%)</td>
<td>34/3543 (8.5%)</td>
<td>0.004</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean length of stay before sample (days; SD)</td>
<td>17.9 (29)</td>
<td>13.6 (23)</td>
<td>11.2 (22)</td>
<td>0.9311</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean length of stay after sample (days; SD)</td>
<td>19.4 (25)</td>
<td>16.5 (24)</td>
<td>15.1 (24)</td>
<td>0.1869</td>
<td>0.0010</td>
</tr>
<tr>
<td>Death rate per 1000 inpatient days</td>
<td>9.03</td>
<td>6.04</td>
<td>6.05</td>
<td>0.0317</td>
<td>0.0018</td>
</tr>
</tbody>
</table>

Table 3: Clinical characteristics of first episodes of inpatients with available clinical outcome results with use of the result of the CTA and NAAT tests to define diagnostic categories

- CCNA + associated with higher death rate, higher WBC
- PCR+ with CCNA negative similar outcomes to all both tests negative
Two Step Testing for *C. difficile*

- PPV relative to either reference method lowest with GDH – NAAT: commonly employed in Canada
- Use of NAAT alone leads to over-diagnosis of CDI relative to CTA
Two Step Testing for *C. difficile*: Considerations

Tests for Presence of *C. difficile*
- Glutamate dehydrogenase (GDH)
- Toxigenic culture
- PCR (*tcdc* gene for toxin)

Tests for Free Toxin
- Cell Culture Cytotoxin Assay (CTA)
- EIA for toxin

**GDH and PCR are similar tests**
Halton Healthcare (“UK Approach”) to C. difficile Testing since May 2015

Key Considerations:

• Prompt CDI reporting to help with bed allocation / IPC decisions.

• Limited lab staffing despite increased operating hours (0600 – 2200h): need for automation

Goals:

➢ Exclude CDI using excellent sensitivity and NPV of PCR

➢ All positive PCR “confirmed” by Ag/Toxin
## Weekday Halton Healthcare BD MAX Workflow

<table>
<thead>
<tr>
<th>Time of Run</th>
<th>Assay</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>630AM</td>
<td><em>C. difficile</em> EBP</td>
<td>BD BD</td>
</tr>
<tr>
<td>10 – 11AM</td>
<td>Flu A/B, RSV</td>
<td>R-BioPharm</td>
</tr>
<tr>
<td>Noon</td>
<td><em>C. Difficile</em> EBP</td>
<td>BD BD</td>
</tr>
<tr>
<td>6PM</td>
<td><em>C. difficile</em> EBP</td>
<td>BD R-BioPharm</td>
</tr>
<tr>
<td></td>
<td>Flu A/B, RSV</td>
<td>BD</td>
</tr>
</tbody>
</table>
HHS C. difficile Testing Reporting Algorithm

1. PCR+, GDH+, EIA +: “Presence of free toxin by EIA indicates infection”

2. PCR+, GDH+, EIA -: “Absence of free toxin by EIA favours colonization; clinical correlation required”

3. PCR+, GDH-, EIA +/ -: “May represent a false positive PCR result; clinical correlation required”
Returning to the Case…

Does this change management?

09/29 @2045h

Campylobacter sp. + by PCR (BD MAX EBP)
54F Case Evolution 09/30

• Seen by Internist at 0300h on 09/30 and started on Ciprofloxacin 400mg IV q12h and Metronidazole 500mg IV q12h

• Reviewed by ID at 1255h: all treatment stopped and patient sent home

• Full recovery to baseline thereafter
Another Case (ca. 2013)...

32F with post partum fever and new onset diarrhea
Case (ca. 2013)

- Schoolteacher, born in Canada, no foreign travel
- Prior Sz disorder; only recent medication is lamotrigine
- No prior medical history or healthcare access until delivery on January 13th, 2013 at 0750 HHS
- Breech presentation  C-section
- Given cefazolin 1g IV x 1 dose during C-section
- No post-operative complications. Infant term and doing well
Case (cont’d)

- January 15th, 2 days post partum: fever (37.9°C) and discharge cancelled
- New onset diarrhea, without mucous or blood
- Concomitant nausea and vomiting; some cramps
- Multiple visitors / family members all well
- U/A normal; Urine C/S negative
- WBC 12.4
Stool for *C. difficile* Cytotoxin PCR POSITIVE

1. Should she be isolated and should she be treated for CDI?
2. More investigations?
Case

Minimal Risk Factors for *C. difficile* Present

- Prolonged hospital stay
- Antibiotic exposure (any drug, rarely short duration)
- Advanced age >65
- Cancer chemotherapy
- PPI use
- ESRD

Case Evolution (ca. 2013)

- Sent home on a full course of metronidazole
- Ten days post discharge: **Rotavirus + by EM**

*We can figure this out faster in 2016...*
Another Case (ca. 2016)...

80F admitted 09/21@1610h with fever and confusion
80F admitted 09/21

- Living independently, no foreign travel, minimal time outdoors, no recent health care access.
- Prior CVA, HTN, CKD, GERD, DM, multiple joint replacements; on Plavix
- Low grade fever, increasing somnolence and obtundation PTA; brought in by daughters
- Fevers (Tmax 38.3) and diarrhea staring the day of admission
- No LP could be performed (on Plavix), Blood C/S 09/21 NG
80F admitted 09/22

09/22: Ceftriaxone  ->

09/24: Vancomycin  ->

Stool *Campylobacter sp.* POSITIVE by PCR

09/26: Worsening mental status

09/27: Worsening mental status; Acyclovir and Ampicillin added

09/27: MRI with gadolinium normal
80F admitted 09/22

09/28: Azithromycin po added on 09/28 and ID consultation requested

09/28: ID reviews: all antibiotics stopped

09/30: Extubated; Full recovery of mental status

Prolonged hospital stay until now for other reasons

Possible diagnosis: *Campylobacter*-associated encephalitis / encephalopathy
Clinical Value of Enteric PCR I
Lessons from Cases

• *C. difficile* infection overdiagnosis has emerged in the molecular era.

  • Two step testing for CDI improves PPV of standalone PCR

  • An immediate alternate diagnosis may prevent overtreatment, over-reporting, unnecessary isolation /hospital stay.
Clinical Value of Enteric PCR II

Lessons from Cases

A positive result for an enteric pathogen:
- may precede other positive results
- may justify antibiotic discontinuation, even in complex situations
- may avert antibiotic use for cases of STEC
Part Two

What is Conventional Stool Culture Missing?
NAAT 1 log10 CFU/mL lower than culture
Pathogen obscured/ inhibited
Salmonella PCR+ example

Direct Plating

Selenite Subculture

Hektoen 24 hours incubation
Orange colonies commensal flora

Selenite broth incubated 6 hours,
Subbed to Hektoen subbed and incubated 24 hours
Black (H₂S) colonies = Salmonella
Sorbitol-negative not evident on SMAC

**STEC CHROMagar**
48h incubation: Diffuse mauve colonies Shiga-toxin E.coli. Darker colonies E.coli.

**SMAC Media**
No sorbitol-negative colony evident
PCR+, Culture-negative Examples

Shiga-Toxin positive by PCR  Shiga-Toxin positive by PCR

STEC CHROMagar incubated for 48 hours
Blue colonies are commensal flora
No mauve colonies.

Selenite subbed after 6 hours incubation
Hektoen incubated 24 hours
Tiny green colonies were not Shigella sp.
ProGastro SGSS vs. Conventional Culture

- Overall positivity rate increased from 5.6% to 8.3%
- Resolution of discrepant results using bidirectional sequencing
• 785 preserved retrospective and 3457 unpreserved prospective stool specimens
• Positive predictive agreement (PPA) > 97.3%; better sensitivity than conventional culture
• Discrepant results resolved by an alternate PCR method and bidirectional sequencing

| TABLE 5 Discrepant results for prospective samples positive by BD Max EBP<sup>a</sup> | No. of BD Max EBP-positive specimens with result |
|---|---|---|
| Target | Culture negative | EIA negative | Alternate PCR positive |
| Campylobacter | 51 | NA | 22 |
| Salmonella | 26 | NA | 19 |
| Shigella | 10 | NA | 9 |
| Shiga toxin | NA | 17 | 9 |

<sup>a</sup> NA, not applicable.
Part Three

Implications of molecular testing on workflow
## Platforms Compared

<table>
<thead>
<tr>
<th></th>
<th>ProGastro</th>
<th>BD MAX</th>
<th>Verigene</th>
<th>xTAG</th>
<th>Biofire</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>Sample to</strong></td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td><strong>Result</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></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 Cycle capacity</td>
<td>Batch 24/run More than one assay type</td>
<td>1 sample/run/instrument</td>
<td>Batch / Limited capacity</td>
<td>1 sample/run/instrument</td>
</tr>
<tr>
<td><strong>TAT</strong></td>
<td>3 hr</td>
<td>2 hr</td>
<td>2 hr</td>
<td>4 hr</td>
<td>1 hr</td>
</tr>
<tr>
<td><strong>Cost/test</strong></td>
<td>$ + extraction</td>
<td>$*</td>
<td>$$ $$</td>
<td>$$ $$</td>
<td>$$ $$</td>
</tr>
</tbody>
</table>
# Performance

<table>
<thead>
<tr>
<th></th>
<th>ProGastro</th>
<th>BD MAX</th>
<th>Verigene</th>
<th>xTAG</th>
<th>Biofire</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>97 - 100%</td>
<td>97.5 –</td>
<td>Variable</td>
<td>91 – 99% for All</td>
<td>95% overall Norovirus</td>
</tr>
<tr>
<td></td>
<td>Salmonella</td>
<td>100%</td>
<td>targets</td>
<td>All targets</td>
<td>C difficile 92%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All</td>
<td></td>
<td></td>
<td>Aeromonas 24%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>targets</td>
<td></td>
<td></td>
<td>Shigella 92%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Adeno 90%</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>99%</td>
<td>99%</td>
<td>99%</td>
<td>99%</td>
<td>99%</td>
</tr>
<tr>
<td><strong>Issues</strong></td>
<td>Carriage or Infection?</td>
<td>Carriage or Infection?</td>
<td>Variable sensitivity of targets</td>
<td>Detect dual infection or is it past infection or carriage?</td>
<td>Detect dual infection or is it past infection or carriage?</td>
</tr>
</tbody>
</table>
Workflow: Narrow Spectrum Panels

**ProGastro SSCS**
- Perform extraction using SpecificA 1.0.2 protocol
- 85 minutes in SmartCycler
- 5 µl of nucleic acid for SSC PCR reaction
- 5 µl of nucleic acid for STEC PCR reaction

**Targets:** Salmon, Shig, Campy, stx1, stx2
**Batch:** Dependent on extractor/SmartCycler
**TAT:** ~4 hours
  Manual set-up, 2 reactions/specimen

**BD MAX EBP**

**Targets:** Salmon, Shig, Campy, stx1, stx2
**Batch:** 1-24
**TAT:** ~2 hours
Automated, sample to answer
Processing: 300-305
## BD MAX IVD Assays in Development

<table>
<thead>
<tr>
<th>ASSAY</th>
<th>TARGETS</th>
<th>ESTIMATED LAUNCH DATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA</td>
<td>MRSA</td>
<td>Available Now</td>
</tr>
<tr>
<td>C. difficile</td>
<td>Toxin B gene (tcdB)</td>
<td>Available Now</td>
</tr>
<tr>
<td>CRE (RUO)</td>
<td>Antimicrobial resistance genes: KPC, OXA-48 and NDM</td>
<td>Available Now</td>
</tr>
<tr>
<td>StaphSR</td>
<td>MRSA and S.aureus (SA)</td>
<td>Available Now</td>
</tr>
<tr>
<td>MRSA XT</td>
<td>Broader range of MRSA strains (mecC, mecA dropouts, new MREJ types).</td>
<td>Available Now</td>
</tr>
<tr>
<td>Enteric Bacterial (EBP)</td>
<td>Salmonella spp., Campylobacter spp. (jejuni / coli), Shigella spp./EIEC, STEC – Shiga toxin producing E. coli.</td>
<td>Available Now</td>
</tr>
<tr>
<td>GC Confirmation</td>
<td>Neisseria gonorrhoeae (confirmatory of positive samples from Probetec Qx assay)</td>
<td>Available Now</td>
</tr>
<tr>
<td>GBS</td>
<td>Group B Streptococcus</td>
<td>Available Now</td>
</tr>
<tr>
<td>Enteric Parasite</td>
<td>Giardia lamblia, Cryptosporidium (parvum and hominis), Entamoeba histolytica</td>
<td>Available Now</td>
</tr>
<tr>
<td>CT/GC/TV</td>
<td>Chlamydia trachomatis , Neisseria gonorrhoeae, Trichomonas vaginalis</td>
<td>Available Now</td>
</tr>
<tr>
<td>Vaginitis/ Vaginosis Panel*</td>
<td>Trichomonas vaginalis; Candida species: C. glabrata, C. krusei; BV: Lactobacillus spp, Gardnerella vaginalis, Atopobium vaginae, BVAB-2, Megasphaere</td>
<td>Winter 2017</td>
</tr>
<tr>
<td>Extended Enteric Bacterial* (MM format for EBP)</td>
<td>Yersina enterocolitica, ETEC, Plesiomonas, Vibrio spp.</td>
<td>Summer 2017</td>
</tr>
<tr>
<td>Enteric Viral*</td>
<td>Norovirus GI/II, Rotavirus A (subtype 1-4), Adenovirus 40/41, Sapovirus/ Astrovirus</td>
<td>Fall 2017</td>
</tr>
</tbody>
</table>

* Product in development. Not currently available in Canada. Subject to approval by Health Canada

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Workflow: Broad Spectrum Panels

**Xtag GPP**
- Extract: 45 min.
- PCR: 1.5 h
- Hybridize: 1.5 h
- Detect: 10 min.
- ~4 hrs
- Targets: 11 Bacteria, Virus, Parasite
- Batch: Dependent on equipment
- Manual set-up, offline extraction and pipetting of amplicon

**Verigene EP**
- Add sample to buffer
- Transfer to EP Test
- ~2 hrs
- Targets: 9 Bacteria, Virus
- On Demand: one sample/module
- Automated processing, transfer to reader

**FilmArray GI**
- ~1 hr
- Targets: 22 Bacteria, Virus, Parasite
- On Demand: one sample/module
- Sample-Answer
### Conventional Stool Cultures

<table>
<thead>
<tr>
<th>Description</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Stool Cultures/year</td>
<td>623</td>
</tr>
<tr>
<td>Basic Stool Culture Workup - Media Costs</td>
<td>$4.68</td>
</tr>
<tr>
<td>Cost of Media x 623 stools = 623 x $4.68</td>
<td>$2,915.64</td>
</tr>
<tr>
<td>Cost of Extra Workup supplies - Potential Positives/Positive Cultures</td>
<td>$2,449.15</td>
</tr>
<tr>
<td>Grand Total Cost of Stool Cultures /Year</td>
<td>$5,364.79</td>
</tr>
<tr>
<td>Cost per Conventional Stool Culture</td>
<td>$8.61</td>
</tr>
</tbody>
</table>

### PCR Testing Methodology

<table>
<thead>
<tr>
<th>Description</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost of PCR = $25.00 x 623 = (cost of 1 test before negotiations)</td>
<td>$15,575.00</td>
</tr>
<tr>
<td>Cost of Yersinia culture on Paeds Patients (No current PCR method)</td>
<td>$373.80</td>
</tr>
<tr>
<td>Culture of Positives for PHL Referral for Confirmation = 52</td>
<td>$809.00</td>
</tr>
<tr>
<td>Total Cost of PCR testing, plus Yersinia and Positive Workups/Year</td>
<td>$16,757.80</td>
</tr>
<tr>
<td>Cost per stool specimen by PCR Testing</td>
<td>$26.90</td>
</tr>
</tbody>
</table>

#### Difference PCR vs. Conventional Methodology per Year

$11,393.01

#### Incremental Cost per Specimen by PCR per Year

$18.29
1. Faster Clinical Decision Making
   • Improved turnaround time could allow for earlier discharge as physicians are no longer awaiting stool culture results to explain a patient's illness
   • Improve the care of patients with acute bacterial gastroenteritis through improved turnaround time, “leading to more timely and directed therapeutic intervention”

2. Higher Sensitivity than Conventional Culture

3. Simplicity of Method for Laboratory technologists as compared with conventional culture
4. Potential Infection Control Benefits

- Discontinuation of Infection Prevention & Control precautions in a more timely basis
- Improved use of limited single patient rooms
- Mitigate the inappropriate use of antibiotics
- Aid in epidemiologic investigations i.e. Food Poisoning
- Provision of an alternate diagnosis in cases of *C. difficile* colonization.
Halton Healthcare Microbiology Laboratory
Three hospital sites, 600 beds total, 9000 specimens / month
8.25FTE Seven Days a Week: 0600 – 2200h

Molecular Specimen Average Daily Workflow:
• Three to five *C. difficile* PCR specimens
• two stool culture specimens
• five respiratory virus specimens
# Weekday Halton Healthcare BD MAX Workflow

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<td>6PM</td>
<td>C. difficile EBP</td>
<td>BD</td>
</tr>
<tr>
<td></td>
<td>Flu A/B, RSV EBP</td>
<td>R-BioPharm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BD</td>
</tr>
</tbody>
</table>
**Current HHS Stool Specimen Workflow**

**Future:** *ad hoc* Norovirus / Rotavirus testing

- **Stool specimen for PCR**
  - Test for *C. difficile*
    - Negative
    - **Positive:** Test by EIA for Ag/Toxin
      - Negative: **Positive** for Salmonella, Shigella, Camyllobacter
        - **Positive for stx1 / stx2**
          - **REPORT** to MOH
        - **REPORT** to MOH
    - **Hektoen, Selenite**
      - **Send to PHL if positive**
  - Add CIN if age < 12
  - **Negative**
    - **Positive:** Test by EIA for Ag/Toxin
      - Negative: **Positive** for Salmonella, Shigella, Camyllobacter
        - **Positive for stx1 / stx2**
          - **REPORT** to MOH
        - **REPORT** to MOH
    - **Hektoen, Selenite**
      - **Send to PHL if positive**
  - **STEC CHROMagar**
    - **Send to PHL if positive**
Part Four

Logistics: Verification of a new gold standard
# Validation of Laboratory-Developed Molecular Assays for Infectious Diseases

## TABLE 1. Required performance characteristics with suggested studies needed before implementation of FDA-approved/cleared tests and laboratory-developed tests

<table>
<thead>
<tr>
<th>Performance characteristic (reference(s)) and suggested study</th>
<th>Requirement(s) for:</th>
<th>Laboratory-developed test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reportable range (8), linearity study (for quantitative assays)</td>
<td>5-7 concentrations across stated linear range, 2 replicates at each concn</td>
<td>7-9 concentrations across anticipated measuring range (or 20-30% beyond to ascertain widest possible range); 2-3 replicates at each concn; polynomial regression analysis</td>
</tr>
<tr>
<td>Analytical sensitivity (14, 28, 33), limit-of-detection study</td>
<td>Not required by CLIA, but CAP requires LOD verification for quantitative assays; use 20 data points collected over 5 days</td>
<td>60 data points (e.g., 12 replicates from 5 samples in the range of the expected detection limit); conduct the study over 5 days; probit regression analysis (or SD with confidence limits if LOB studies are used)</td>
</tr>
<tr>
<td>Precision (7, 13, 15, 40), replication experiment</td>
<td>For qualitative test, test 1 control/day for 20 days or duplicate controls for 10 days; for quantitative test, test 2 samples at each of 2 concentrations (4 samples) plus one control over 20 days or test 2 concentrations in triplicate over 5 days</td>
<td>For qualitative test, minimum of 3 concentrations (LOD, 20% above LOD, 20% below LOD) and obtain 40 data points; for quantitative test, minimum of 3 concentrations (high, low, LOD) and test in duplicate 1-2 times/day over 20 days; calculate SD and/or CV within run, between run, day to day, total variation</td>
</tr>
<tr>
<td>Analytical specificity (28), interference study</td>
<td>Not required by CLIA</td>
<td>No minimum no. of samples recommended; test sample-related interfering substances (hemolysis, lipemia, icterus, etc.) and genetically similar organisms or organisms found in same sample sites with same clinical presentation; spike with low concentration of analyte; paired-difference (t test) statistics</td>
</tr>
<tr>
<td>Accuracy (trueness) (13), comparison-of-methods study</td>
<td>20 patient specimens within the measuring interval or reference materials at 2 concentrations (low and high) in duplicate over 2-5 runs</td>
<td>Test in duplicate by both the comparative and test procedures over at least 5 operating days; typically 40 or more specimens; xy scatter plot with regression statistics; Bland-Altman difference plot with determination of bias; % agreement with kappa statistics</td>
</tr>
</tbody>
</table>
Community Hospital Laboratories: Challenges of verification

<table>
<thead>
<tr>
<th>PATHOGEN</th>
<th>IN HOUSE METHOD</th>
<th>OPHL REFERENCE LAB METHOD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella sp.</em></td>
<td>CULTURE (Hektoen, Selenite)</td>
<td>CULTURE</td>
</tr>
<tr>
<td><em>Campylobacter sp.</em></td>
<td>CULTURE (Campylobacter (Karmali))</td>
<td>CULTURE</td>
</tr>
<tr>
<td>stx1, stx2</td>
<td>CULTURE (STEC)</td>
<td>EIA/ [RT-PCR (NML)]</td>
</tr>
<tr>
<td><em>Shigella sp.</em></td>
<td>CULTURE (Hektoen, Selenite)</td>
<td>CULTURE</td>
</tr>
</tbody>
</table>
Enteric Bacterial Panel (EBP) Verification to November 2016

<table>
<thead>
<tr>
<th>EBP PCR RESULT</th>
<th>CONFIRMED IN HOUSE</th>
<th>CONFIRMED REFERENCE LAB</th>
<th>TOTAL CONFIRMED</th>
<th>OPHL REFERENCE METHOD NEGATIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO ORGANISM DETECTED</td>
<td>265 (100%)</td>
<td>0</td>
<td>265 (100%)</td>
<td>N/A</td>
</tr>
<tr>
<td><em>Salmonellas sp.</em> DETECTED</td>
<td>38 (88%)</td>
<td>2 (5%)</td>
<td>40 (93%)</td>
<td>3 (7%)</td>
</tr>
<tr>
<td><em>Campylobacter sp.</em> DETECTED</td>
<td>21 (84%)</td>
<td>2 (8%)</td>
<td>23 (92%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>STEC DETECTED Pre-CHROMagar</td>
<td>2 (25%)</td>
<td>EIA 3 (37.5%)</td>
<td>5 (62.5%)</td>
<td>EIA-negative 3 (37.5%)</td>
</tr>
<tr>
<td>STEC DETECTED Post-CHROMagar</td>
<td>6 (67%)</td>
<td>1 (11%)</td>
<td>7 (78%)</td>
<td>2 (22%)</td>
</tr>
<tr>
<td>SHIGELLA DETECTED</td>
<td>4 (44%)</td>
<td>2 (22%)</td>
<td>6 (66%)</td>
<td>3 (34%)</td>
</tr>
</tbody>
</table>

No culture-positive / PCR negative specimens
Community Hospital Laboratories: Challenges of Verification I

- Gold standard method (alternate PCR, bidirectional gene sequencing) not readily available
- Need to rely on local public health lab protocols which may not use the gold standard
- Few other labs using an identical or alternative molecular method to assist with verification
Community Hospital Laboratories: Challenges of Verification II

- In house conventional culture has low sensitivity: what to do with PCR+ / culture negative isolates?
- Low frequency isolates slow verification
  - *Shigella* and STEC more rarely encountered
  - Use stored specimens or use prospective specimens?
  - Use a recovery matrix and perform analytical sensitivity?
- Need to implement a new non-SMAC method to identify STEC (EIA or CHROMagar) for positive results
- Could the manufacturers provide proficiency panels, especially for rarer isolates?
Community Hospital Laboratories: Validation

1. PCR- / culture-positive raises a red flag
2. PCR- / culture-negative specimens: need EQA
3. PCR+/ culture-positive: partially validate the assay
4. What about PCR+ / culture negative?
   a) Role for OPHL or NML?
   b) Or could the manufacturers fund a few designated reference laboratories in Canada? Who pays for it?
   c) A role for NMG to create a network of users…
HHS Verification and Implementation of EBP

- Prospective specimen verification began August 2015
- In July 2016 started reporting PCR negatives, positive *Salmonella sp.* and *Campylobacter sp.*
  - >200 negative culture/ PCR
  - >40 confirmed *Salmonella sp.*
  - >20 confirmed *Campylobacter sp.*
  - >10 confirmed STEC
  - <10 *Shigella sp.*
- Stopped routine culture of stools in July 2016
- Continue to culture for *Yersinia sp.* in paediatric population
- Continue to perform directed culture for pathogens detected by PCR
- Will spike a few more specimens with *Shigella sp.* before “going live”.
Conclusions: Strengths of Molecular Testing for GI Pathogens in the Community

• Automation: a LEAN approach by sorting out the negatives

• Better sensitivity than conventional testing; STEC now detected

• Immediate results / PH notification

• Potential for early discontinuation of isolation, ?discharge

• Avoidance of unnecessary antibiotics (e.g. *Salmonella*, *Campylobacter*, EHEC) for uncomplicated cases

• Low complexity, especially for negatives
Conclusions: Limitations of Molecular Testing for GI Pathogens in the Community

- Still need to perform conventional culture method for positive results
  - What to do with PCR+ / culture negative?
- Focused panel menu misses some key pathogens
- Comprehensive broad panels costly and too expansive
- Higher cost per specimen than conventional culture, though lower cost per positive.
- Challenges with verification and validation
- LIS interface to avoid manual entry of results
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