The Changing Paradigm of the Laboratory Diagnosis of Gastroenteritis

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Gastrointestinal disease - worldwide

- Over 2 billion cases annually
- Diarrheal disease is the 2nd leading cause of death in children <5 yo.
  - >760,000 pediatric deaths annually
  - Leading cause of malnutrition
- Can be prevented through safe drinking water and improvements in sanitation and hygiene

Cartogram by Worldmapper: number of cases/100,000 children

Gastrointestinal disease – U.S.

- 250,000 hospitalizations
- 8 million physician visits
- 99 million episodes of acute gastroenteritis

Common causes of gastroenteritis

- Bacterial
  - Aeromonas
  - Campylobacter spp.
  - C. difficile
  - E. coli (EIEC, STEC, EPEC, ETEC)
  - Plesiomonas shigelloides
  - Salmonella
  - Shigella
  - Vibrio spp.
  - Yersinia enterocolitica
- Viral
  - norovirus, sapovirus, astrovirus, rotavirus, enteric adenoviruses
- Parasitic
  - Giardia lamblia, E. histolytica, Cryptosporidium, Cyclospora, Isospora

Lab-confirmed gastrointestinal pathogens – U.S.

- Norovirus: ~1.5-2 million cases/yr

Disclosures

- Scientific Advisory Board
  - Cepheid
- Grants
  - Luminex Molecular Diagnostics
  - Becton Dickinson
  - Cepheid

Scallan et al. 2011, EID, 17:7-15
Laboratory Diagnosis of Gastrointestinal Infections

Culture has a wide breadth, but is labor intensive with a longer time to result. Supplementation testing needed to detect STEC and Campylobacter.

Detection of viral agents is largely not performed. LDTs and EM may be available, but at considerable cost.

Parasitic detection involves a combination of antigen/DFA methods and traditional microscopy. Labor intensive, long time to result, and somewhat insensitive.

Detecting Bacterial Agents

• Traditional culture

![Traditional culture image]

Time to result = 2-4 days

Detecting Bacterial Agents

• Shiga toxin detection
  » CDC recommends Shiga toxin detection on all stools
  » Not practical or cost-effective for most institutions

<table>
<thead>
<tr>
<th>Test</th>
<th>Campy toxin (2+)</th>
<th>Cost per positive (USD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shiga toxin</td>
<td>5.00</td>
<td>25.00</td>
</tr>
</tbody>
</table>

TABLE 1. Cost of stool testing: Yerba Malaga Unleashed Hospital

Shiga toxin detection
- Performance of EIA, PCR and SMAC detection
- 21 positive (12 patients) out of 632 stools (430 patients) (3.3%)
  - 21 (100%) positive by PCR (LOD 10^6 cfu/ml)
  - 6 (28.6%) positive by EIA (LOD 10^3-10^4 cfu/ml)
  - 5 (23.8%) positive by SMAC culture (O157)
- No false positives in this study

Detecting Bacterial Agents

• Campylobacter detection

Giltner et al., 2013, JCM 51: 618-620

Detecting Bacterial Agents

Vallières et al., 2013, JCM 51: 481-486

Survey courtesy: Susan Sharp, PhD

Ghosh et al.
The Problem

- Traditional methods are very labor intensive, some have decreased sensitivity, and the turnaround time can be days
- Positivity rates are generally low
  - Bacterial culture: 3.9%
  - Giardia/Cryptosporidium: <0.1%
  - Rotavirus: 0%

- Until recently, no method for diagnosing norovirus

The “Solution”

- Molecular multiplex testing for GI pathogens
  - Clinically indistinguishable syndromes (e.g. gastrointestinal disease)
  - Faster time to result over traditional methods
  - Susceptibility testing generally not needed
  - Potential increased sensitivity
    - Allows for less testing
    - High negative predictive value
  - Cost savings over combination of traditional methods

Test(s) ordered:
- GPP Positive for:
  - C. difficile
  - Campylobacter
  - C. difficile
  - Giardia
  - C. difficile
  - Bacterial culture w/o ST
  - STEC (2)
  - C. difficile
  - CO&P
  - STEC
  - Bacterial culture + ST
  - Giardia
  - Bacterial culture + ST
  - Rotavirus (2)
  - Bacterial culture w/o ST
  - E. coli O157 (1)
  - ETEC (1)
  - Bacterial culture w/o ST
  - Parasite screen
  - E. coli O157 (2)
  - Rotavirus (1)
  - CO&P
  - STEC

Samples positive for analytes for which the physician did not order:

- ST = Shiga toxin
- Parasite screen = Giardia and Cryptosporidium
- CO&P = Comprehensive parasite exam

FDA-cleared multiplex gastrointestinal tests

Comparison of workflow, throughput, and turnaround time among three commercial, FDA-cleared multiplex GI platforms.

Comparison of 2 multiplex panels


Khare et al., 2014 J Clin Microbiol. 52:3667-3673
Implementation of molecular GI panel

- Implemented clinically July 2013
- Test performed daily, M-F
- Discontinued
  - Bacterial stool cultures
  - Parasite screens (Giardia/Cryptosporidium)
  - Ova and parasite microscopy still offered for other parasites.
  - Rotavirus antigen
- Separate tests/orders
  - C. difficile 2-step algorithm
  - Adenovirus stool PCR
  - Norovirus RT-PCR (NEW!)

Ova and parasite microscopy still offered for other parasites.
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Specimens not accepted if onset of diarrhea is >3 days after admission
- Cannot use as test of cure
- 1 test per patient episode

Transport to lab <2h
Transport to lab ≥2h

Luminex xTAG GPP

- Run of 21 samples
- Hands-on time: 90 min
- Time to result: 5.5 h

Luminex xTAG GPP

- Verification: 175 specimens
  - 99 concordantly negative
  - 76 positive for 1 or more analyte(s) = 89 positive results

Summary of concordant samples tested by xTAG GPP

<table>
<thead>
<tr>
<th>UNC Samples</th>
<th>NCSLPH Samples</th>
<th>Total Positives</th>
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<tbody>
<tr>
<td>Campylobacter</td>
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<td>8</td>
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<td>Cryptosporidium</td>
<td>E. coli O157</td>
<td>1</td>
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<td>E. coli O157</td>
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<td>Salmonella</td>
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Luminex xTAG GPP

The 2x2 table illustrates the consensus positive (60), negative (99) and discordant results.

<table>
<thead>
<tr>
<th>Traditional Method</th>
<th>GPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Sensitivity: 90.9%</td>
<td>Overall Specificity: 93.4%</td>
</tr>
</tbody>
</table>

UNC, unpublished data

Advantages and Challenges of Multiplex Molecular GI Testing

Advantages
• Increased detection of viral gastroenteritis

Advantages
• Increased sensitivity and negative predictive value

Advantages
• Increased number of positives and co-detections

Advantages
• Decreased time to result
• Increased detection of secondary transmission events and reportable disease


Advantages

• Reduction in per patient testing

Monthly Average Pre-GI Panel

Monthly Average Post-GI Panel

Advantages

• Provides robust surveillance data to clinical staff
  » May allow for clinical/epidemiologic diagnosis without testing

Challenges

• Verification Study – limited positives
• To report C. difficile or not?
• Off label specimen types – Cary-Blair/straight stool/colostomy bag
• Increased inhibition/repeat rate
  » Remember to account for this in your cost analysis
• Cannot be used as test of cure
• Discontinuation of culture and impact on public health

Challenges

• Maintenance of methods for detecting bacteria not on a panel
  » Depending on volume and geographic incidence, consider sending to a reference lab

Organizer | N | Positivity Rate
--- | --- | ---
Entamoeba species | 5 | 0.21
Fungi parahaemolytica | 1 | 0.07
Syedella spp. | 2 | 0.14
Aquaculture species | 2 | 0.13

• Support for public health needs
  » Consider culturing bacteria+ specimens to obtain an isolate
    » If the stool is not received in Cary-Blair, we inculcate one for positive speciments.
  » Shipping stool to public health labs – Communicate with your local/state public health lab PRIOR to implementation!

Advantages

• Cost savings

<table>
<thead>
<tr>
<th>Table</th>
<th>Description of conventional and GI panel testing pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of isolation days</td>
<td>1255</td>
</tr>
<tr>
<td>Total isolation costs</td>
<td>$19,033</td>
</tr>
<tr>
<td>Total GI panel testing costs</td>
<td>$5,465</td>
</tr>
<tr>
<td>Total costs</td>
<td>$24,498</td>
</tr>
<tr>
<td>GI panel testing pathway</td>
<td></td>
</tr>
<tr>
<td>Total number of isolation days</td>
<td>1497</td>
</tr>
<tr>
<td>Total isolation costs</td>
<td>$21,708</td>
</tr>
<tr>
<td>Total GI panel testing costs</td>
<td>$6,194</td>
</tr>
<tr>
<td>Total costs</td>
<td>$28,002</td>
</tr>
<tr>
<td>Difference (GI panel testing pathway – conventional testing pathway)</td>
<td></td>
</tr>
<tr>
<td>Total number of isolation days</td>
<td>-242</td>
</tr>
<tr>
<td>Total isolation costs</td>
<td>-2,673</td>
</tr>
<tr>
<td>Total GI panel testing costs</td>
<td>-729</td>
</tr>
<tr>
<td>Total costs</td>
<td>-8,142</td>
</tr>
</tbody>
</table>

* Includes confirmatory culture and environmental sampling

At UNC:
GI panel $93 less expensive than combination of traditional methods

» Primarily labor savings
» More sensitive detection = less repeat tests

Challenges

• Specificity
  » Presumptive positive disclaimer: “xTAG GPP positive results are presumptive and must be confirmed by FDA-cleared tests or other acceptable reference methods.”
  » Cost-effectiveness depends on discontinuation of other methods.

• Example: Giardia (PI: >250 is positive)
  » MFI <500 = NEGATIVE
  » MFI ≥500 = repeat the assay and send an aliquot to Microbiology for Giardia DFA (no billing/no reporting)
    » If DFA positive, report POSITIVE
    » If DFA negative but MFI ≥1000, report as INDETERMINATE
Challenges

• Clinically-relevant reporting
  » Example: norovirus shedding
  » Consider verifying and establishing an indeterminate range for "low" positives, or verify internal positivity threshold
  » Add comment to report to aid in interpretation
    • "Norovirus can shed in the stool of healthy individuals for up to a month. In immunocompromised individuals, norovirus shedding has been demonstrated for several months to over a year."

<table>
<thead>
<tr>
<th>Probe MFI Report</th>
<th>GI or GII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>&lt;200</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>200-799</td>
</tr>
<tr>
<td>Positive</td>
<td>&gt;800</td>
</tr>
</tbody>
</table>

Challenges

• Reimbursement
  » CPT code: 87506 x 1 (Infectious agent detection by nucleic acid (DNA or RNA); gastrointestinal pathogen (eg, Clostridium difficile, E. coli, Salmonella, Shigella, norovirus, Giardia), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 6-11 targets)
  » 2016 Clinical Diagnostic Laboratory Fee Schedule
    - Michigan: $290.74
    - NC: $147.76

No Test is Perfect: Case Example

Case Example

• He has a couple of loose stools per day.
  » No nausea or vomiting.
  » Clinician suspected HIV related diarrhea or possibly disseminated M. avium infection
• Patient returned to clinic on 7/30 with continued diarrhea
  » Three loose stools/day, mainly at night or in the early mornings.
  » He describes them as greenish in color.

Case Example

• Molecular GI Pathogen Panel was performed

Case Example

• A 22 yo male was seen in our ID clinic to establish care on 7/2 after being diagnosed with HIV two weeks prior
• He had a ~3-month history of diarrhea, fatigue, weight loss (30 lbs).
  » He was previously admitted to an OSH with a diagnosis of C. difficile colitis where he was treated.
  » He presented to UNC after treatment with worsening diarrhea and was admitted for 3-4 days. He received fluconazole and trimethoprim/sulfamethoxazole and was discharged to follow up in clinic.
  » CD4 count was 59/ul and HIV viral load 12,800 cp/ml
  » Stool culture was negative on admission as was O&P and C. difficile testing.
Case Example

- The next day the patient was prescribed 5d ciprofloxacin for his Campylobacter infection.
  - Patient’s symptoms slightly improved on cipro, but had diarrhea again on day 5 which continued over the next 10 days and led to a 6 lb weight loss.
  - He denies any blood or mucus in his stool.
- The physician called me about:
  1. Testing for cipro resistance in his Campylobacter.
  2. Re-testing his stool via GPP for a “test of cure”

Case Example

- Campylobacter and ciprofloxacin
  - Resistance is being reported with increasing frequency in the U.S.
  - No ciprofloxacin resistance among C. jejuni human isolates in 1990 but since 2001 ~20% of isolates are ciprofloxacin resistant.
  - Only 1.7% of isolates were erythromycin resistant in 2006, and 46% were tetracycline resistant.
  - In lieu of isolating organism (unlikely), clinician chose to treat with a macrolide.
- “Test of cure”
  - I convinced her this was of no use, and we cancelled the test. However… the lab is way too efficient and the stool was already on the run.

Case Example

- Second GPP run

Case Example

- Confirmed with modified acid fast stain and DFA
Case Example

So why is the Cryptosporidium target on the GPP negative?

Summary

- The use of multiplex GI tests is emerging.
- One size does not fit all… determine which testing algorithm is best for your institution/patients.
- Initial cost analyses and projected laboratory and patient impact should be re-assessed post-implementation.
- Communication with our public health partners is key!

Thank you! Questions?
Melissa.Miller@unchealth.unc.edu