Molecular Detection of Gastrointestinal Pathogens

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Acute Diarrheal Illness

• Significant morbidity and mortality:
  – More significant in developing nations
  – Prevent dehydration, provide rehydration

• Most acute GI infections are not reported or intervened medically in the United States.¹

• CDC estimates >350 million acute diarrheal illnesses annually.²

• FoodNet reports 48 million are foodborne.

What do we routinely test for?

**Bacteria**

**Parasites**

**Viruses**
Testing for GI Pathogens

What is the actual prevalence?

- **Viruses**
- **Bacteria**
- **Parasites**
Facts About GI Pathogens

1. Viruses—most prevalent; least tested\(^1\)
   - Norovirus is the number one GI infection in the U.S.

2. Bacteria—stool culture = most common test
   - Only positive in 1–5% of specimens.\(^2\)

3. Parasites—ova and parasite exam = overused/misused test
   - Domestically acquired infections are typically associated with defined exposure risks.

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How Can Multiplex Molecular Detection Help?

• Syndromes may be too similar to separate clinically.
  – Lack of standardized/differential driven ordering by physicians:
    ✓ Too many cultures
    ✓ Too many O&Ps
    ✓ No (or little) viral testing

• Provides faster, more sensitive and specific results for patients.

• Reduces burden on laboratories:
  – Allows for consolidation of redundant testing, reduces wasteful testing.
FDA Cleared Testing Approaches

- Prodesse ProGastro SSCS
- BD Max Enteric Bacterial Panel & Enteric Parasite Panel
- Nanosphere Verigene Enteric Pathogen test
- Luminex xTAG Gastrointestinal Pathogen Panel (GPP)
- Biofire Diagnostics FilmArray GI panel
Prodesse Pregastro SSCS

- Open platform, bacteria only
- Real-time PCR
- Extraction: Biomerieux NuclisSENS easyMAG system
- Amplification: Cepheid Smart Cycler II

- Salmonella
- Shigella/EIEC
- Campylobacter
- Shiga-like toxin producing E. coli (STEC) stx1/stx2
BD Max Enteric
Enteric Bacterial & Parasitic Panels

- All-in-one platform
- “Walkaway” PCR
- Integrated extraction and amplification

Parasitic Panel
- Giardia
- Cryptosporidium
- Entamoeba histolytica

Bacterial Panel
- Salmonella
- Shigella/EIEC
- Campylobacter
- Shiga-like toxin producing E. coli (STEC) stx1/stx2

www.bd.com
Bacteria

- *Campylobacter* spp.
- *Salmonella* spp.
- *Shigella* spp.
- *Vibrio* spp.
- *Yersinia enterocolitica*
- Shiga toxin 1 and 2

Viruses

- Norovirus
- Rotavirus

http://www.nanosphere.us/product/enteric-pathogens
Verigene Enteric Pathogens

- Cartridge format
- Real-time PCR
- Hybridization to array
- Hybridization to oligonucleotide + gold particles
- Signal amplification with silver particles
- Detection by light scattering on array

http://www.nanosphere.us/product/enteric-pathogens
Verigene Enteric Pathogens

✓ Most infections are viral.
✓ Most testing is for bacteria.

• Sweet spot?
• Broad panel in development (+ parasites)
• Option to bill by reportable? (“flex” model)
  – Only pay for what you test.

http://www.nanosphere.us/product/enteric-pathogens
Luminex xTAG GPP

Bacteria
- Salmonella
- Shigella/EIEC
- Campylobacter
- Clostridium difficile Toxin A/B
- Enterotoxigenic E. coli (ETEC) LT/ST
- E. coli O157
- Shiga-like toxin producing E. coli (STEC) stx1/stx2

Viruses
- Rotavirus A
- Norovirus GI/GII
- Adenovirus 40/41

Parasites
- Giardia
- Cryptosporidium
- Entamoeba histolytica

https://www.luminexcorp.com/clinical/infectious-disease/gastrointestinal-pathogen-panel/
Luminex xTAG GPP

Multiplex PCR → Primer extension with xTAG → Tag hybridized to anti-tag coupled xMAP beads

Hybridized beads read and analyzed

https://www.luminexcorp.com/clinical/infectious-disease/gastrointestinal-pathogen-panel/
FilmArray GI Panel

Bacteria
✓ ETEC
✓ EPEC
✓ STEC/EHEC
✓ STEC 0157 serotype
✓ EAggEC
✓ Vibrio spp.
✓ Shigella spp./EIEC
✓ Salmonella spp.
✓ Campylobacter spp.
✓ Yersinia enterocolitica
✓ Clostridium difficile
✓ Plesiomonas shigelloides

Viruses
✓ Norovirus (GI, GII, GIV)
✓ Adenovirus F 40/41
✓ Rotavirus (A, B, C)
✓ Astrovirus
✓ Sapovirus

Parasites
✓ Cryptosporidium spp.
✓ Giardia lamblia
✓ Entamoeba histolytica
✓ Cyclospora cayetanensis

http://filmarray.com/the-panels/
Pros of Molecular GI Testing

• Reduce turnaround (volume and method dependent).
• Replace cumbersome cultures.
• Redirect FTEs to other testing:
  – Replace retiring microbiologists with “generalist” microbiology.
• Replace less sensitive tests entirely:
  – Culture, antigen, microscopy
• Increase sensitivity for challenging organisms:
  – e.g., Campylobacter, STEC, parasites
• Detect organisms not tested for previously.
Case Examples

72 y.o. female w/pmh colon Ca.

- Several weeks of diarrhea, typically after eating, 2–3 loose stools/day (May–June 2015)
- Explosive, loose, voluminous stool, preceded by intense cramps
- Chills and sweats, no fevers
- From Utah; no travel Hx, no antibiotic use
- Concern for salads she began eating regularly in recent weeks

69 y.o. male w/pmh IBS (x26 yrs)

- 6–7 week h/o diarrhea, 3–4 watery stools/day (May–June 2015)
  - Patient noted “different than IBS”
- No recent travel (lives in Utah) or antibiotic use
- Fecal lactoferrin (+) (consistent w/IBS)
- Fecal occult blood (-)
- C. difficile PCR (-)
Diagnosis: Cyclosporiasis

*Cyclospora cayetanensis*
(First cases from 2015 national outbreak)

• Neither physician had suspected *Cyclospora*, but suspicion of endemic parasites prompted GI parasite PCR:
  – One physician was not familiar with *Cyclospora* or that it needed to be treated very specifically.
  – Conventional modified acid-fast stain was not ordered by these clinics in prior years.

• Diagnosis would have typically been missed = underdetection.
Cons of Molecular GI Testing

• Capital expenditures required

• Billing/reimbursement challenges

• Multiple analytes tested when only one may be suspected (or others not relevant):
  – e.g., swimming pool = Cryptosporidium, parasite ≠ long-term inpatients

• Detect organisms that may not be cause of symptoms:
  – Norovirus and Salmonella = prolonged shedding
  – Clostridium difficile = asymptomatic colonization
  – May result in increased calls to the lab

• May not allow culture if required for antibiotic susceptibility testing or outbreak investigations
Molecular Testing Considerations

• Not appropriate in every patient:
  – Lab must educate providers on appropriate use (i.e., every patient doesn’t just “get the test”).
    • Cannot let this testing become the “new O&P.”
  – Consider listing price of test in CPOE.

• Will results influence clinical care?
  – Most viral/bacterial infections are self-limiting.

• Should broad/syndromic panels be SOP for your laboratory?
Molecular Testing Considerations

• Is turnaround time fast enough to influence care decisions? (batch vs. random access, lab capacity for flux volumes)

• Positive result = stop adjunct testing = reduce lab resource waste?

• A test and answer = excellent patient experience?
  – Depends on the cost
Molecular Testing Cost Considerations

- Cost may be significant limiting factor:
  - Who pays for this (outpatients)?
    - Can lab budget absorb these expenses if necessary?
    - What if public health mandate cultures be maintained for outbreaks?
  - CPT codes released 2015:
    - 87505, 3–5 targets
    - 87506, 6–11 targets
    - 87507, 12–25 targets
  - To date, rates are not clearly established.
Take-Home Points

• Gastrointestinal illnesses are one of the most common infections in the U.S.

• Molecular multiplex GI testing can positively impact:
  – Patients
  – Laboratories
  – Public health and safety

• Commercial tests are available in varying formats, turnaround time, and throughput.

• Cost may be a significant barrier: think carefully how this will work in your lab/hospital (look before you leap).

• Utilization efforts will be needed and must include laboratory staff and physicians.