Molecular Detection of Gastrointestinal Pathogens

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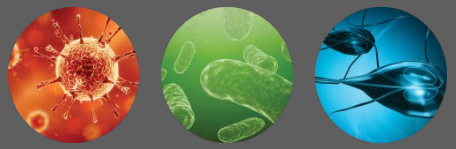
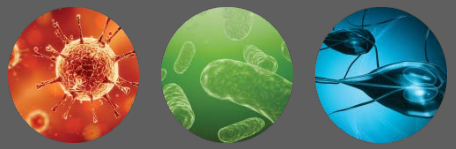
Acute Diarrheal Illness



- 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.

[1]Graves. *Prim Care Clin Office Pract* 2013;40:727–41.
[2] Mead et al. *Emerg Infect Dis* 1999;5:607.

Testing for GI Pathogens

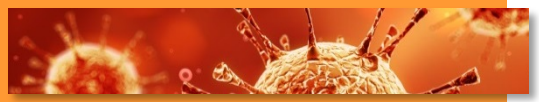


What do we routinely test for?

Bacteria

Parasites

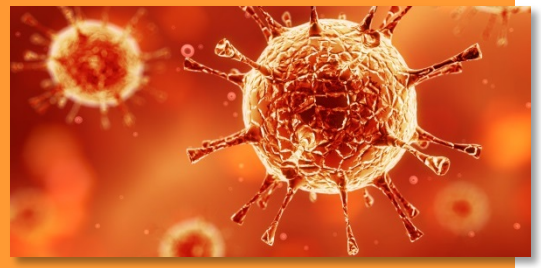
Viruses



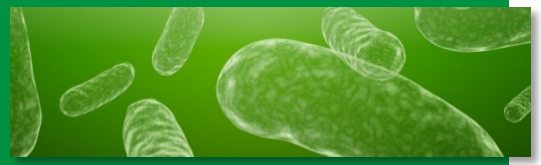
Testing for GI Pathogens

What is the actual prevalence?

Viruses

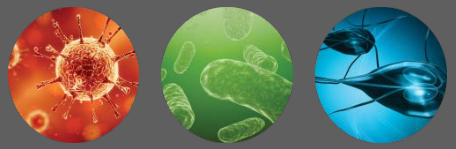


Bacteria



Parasites

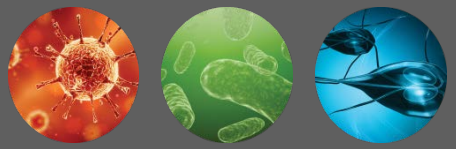
Facts About GI Pathogens



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.

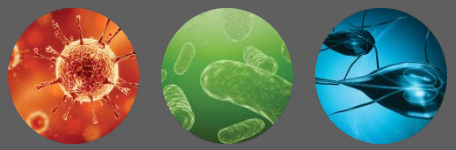
[1]Guerrant et al. *Clin Infect Dis* 2001;32:337–8.
[2]Graves. *Prim Care Clin Office Pract* 2013;40:727–41.

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



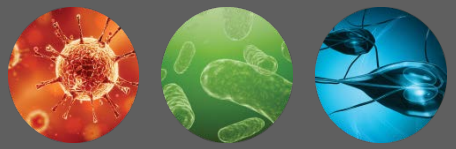
- Prodesse ProGastro SSCS
- BD Max Enteric Bacterial Panel & Enteric Parasite Panel
- Nanosphere Verigene Enteric Pathogen test
- Luminex xTAG Gastrointestinal Pathogen Panel (GPP)
- Biofire Diagnostics FilmArray GI panel

Prodesse Progastro SSCS

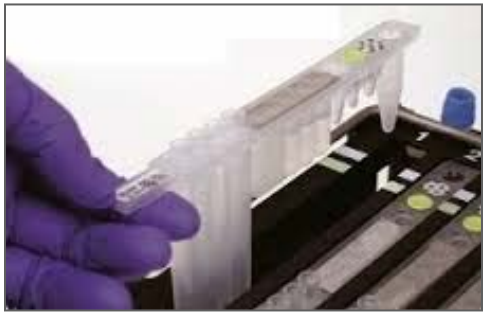
- Open platform, bacteria only
- Real-time PCR
- Extraction: Biomerieux NucliSENS easyMAG system
- Amplification: Cepheid Smart Cycler II

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

BD Max 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

Verigene Enteric Pathogens

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

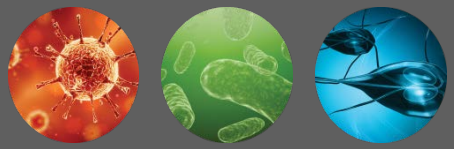
- ✓ Rotavirus A
- ✓ Norovirus GI/GI
- ✓ Adenovirus 40/41

Parasites

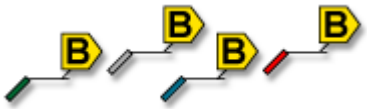
- ✓ *Giardia*
- ✓ *Cryptosporidium*
- ✓ *Entamoeba histolytica*

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

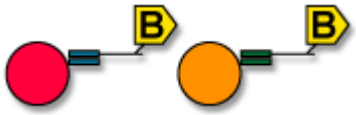
Luminex xTAG GPP



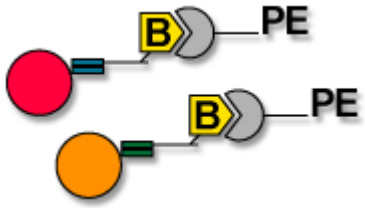
Multiplex PCR



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

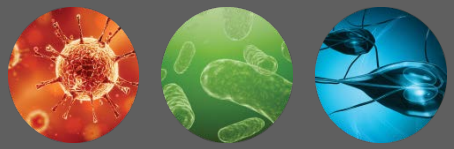
- ✓ Norovirus (GI, GII, GIV)
- ✓ Adenovirus F 40/41
- ✓ Rotavirus (A, B, C)
- ✓ Astrovirus
- ✓ Sapovirus

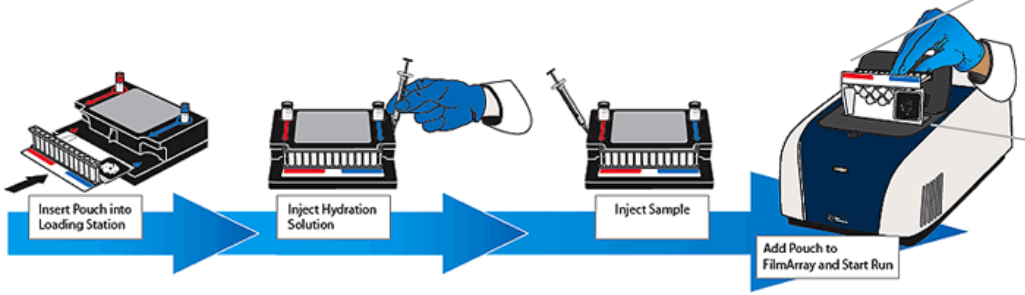
Parasites

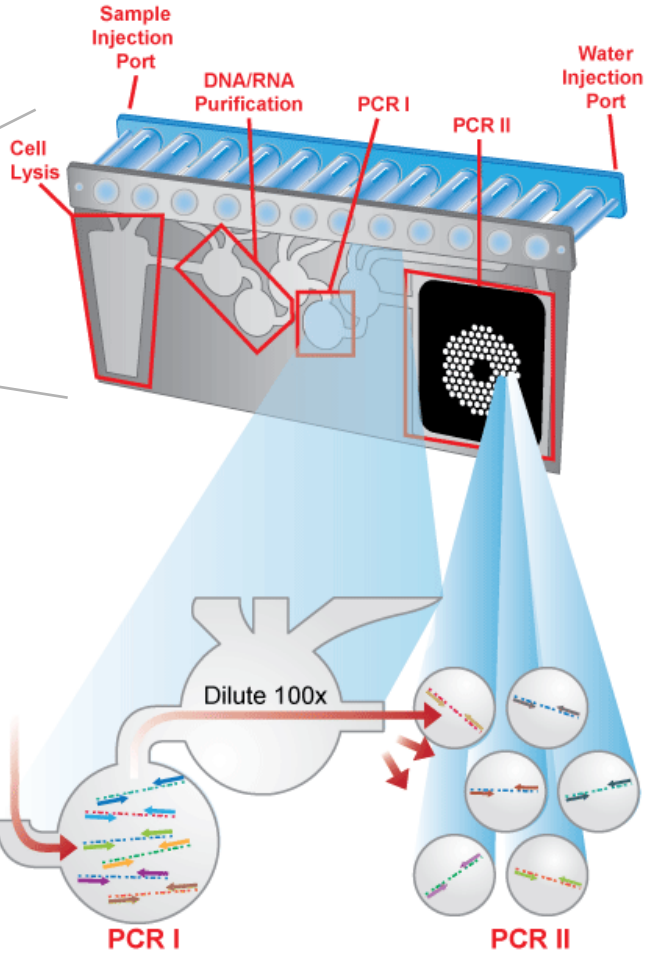
- ✓ *Cryptosporidium* spp.
- ✓ *Giardia lamblia*
- ✓ *Entamoeba histolytica*
- ✓ *Cyclospora cayetanensis*

http://filmarray.com/the-panels/

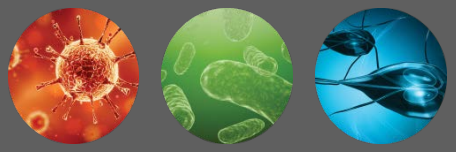
FilmArray GI Panel





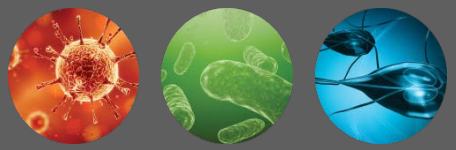


Pros of Molecular GI Testing



- 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

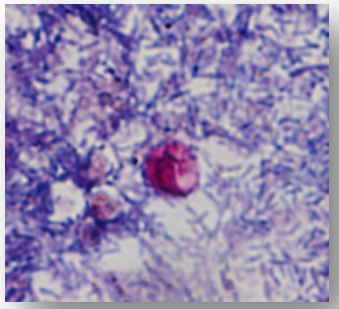
69 y.o. male w/pmh IBS (x26 yrs)

- 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 (-)

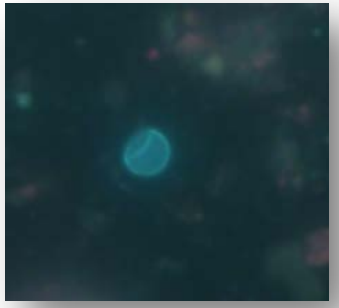
Diagnosis: Cyclosporiasis

Cyclospora cayetanensis

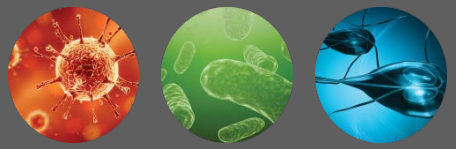
(First cases from 2015 national outbreak)



- 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.

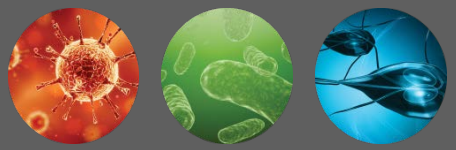


Cons of Molecular GI Testing



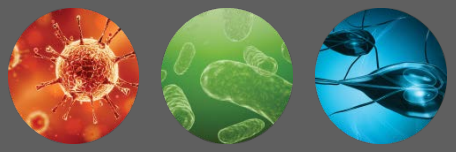
- Capital expenditures required
- Billing/reimbursement challenges
- Multiple analytes tested when only one may be suspected (or others not relevant):
  - e.g., swimming pool = *Cryptosporidium,* parasite ≠ long-term inpatients
- Detect organisms that may not be cause of symptoms:
  - Norovirus and *Salmonella* = 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



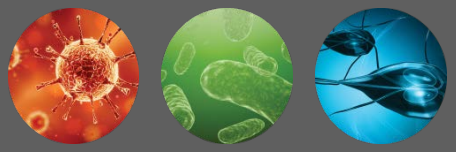
- Not appropriate in every patient:
  - Lab must educate providers on appropriate use (i.e., every patient doesn't just "get the test").
    - Cannot let this testing become the "new O&P."
  - Consider listing price of test in CPOE.
- Will results influence clinical care?
  - Most viral/bacterial infections are self-limiting.
- Should broad/syndromic panels be SOP for your laboratory?

Molecular Testing Considerations



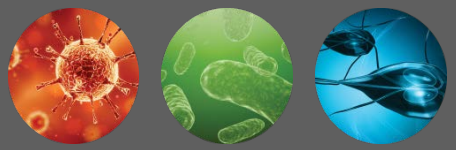
- Is turnaround time fast enough to influence care decisions? (batch vs. random access, lab capacity for flux volumes)
- Positive result = stop adjunct testing = reduce lab resource waste?
- A test and answer = excellent patient experience?
  - Depends on the cost

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.