Multiplex/molecular testing for gastrointestinal infections

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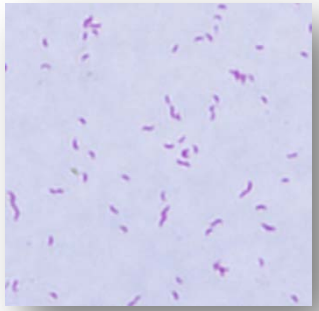
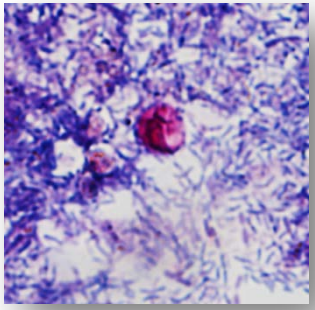




Objectives

1. Understand the traditional approaches to gastroenteritis testing (Parasitology skew)

2. Compare and contrast the available multiplex molecular diagnostic assays for gastroenteritis

3. Discuss test utilization of multiplex molecular diagnostics for gastroenteritis

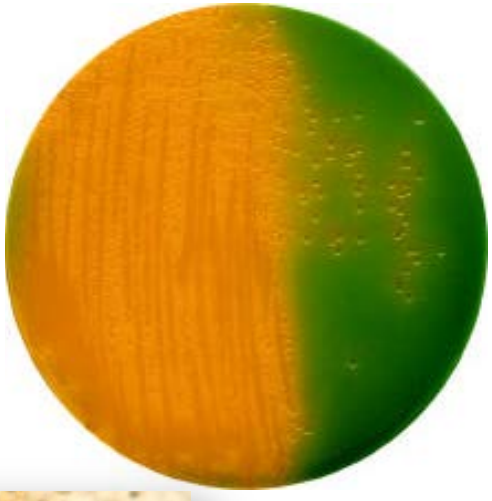
Disclosures

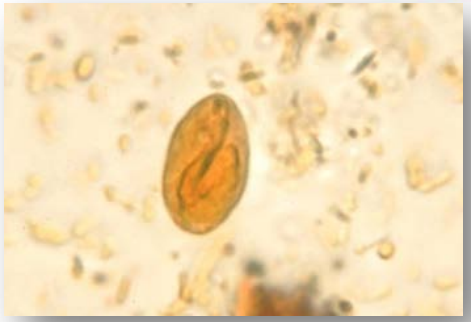
- Research reagents
  - BioFire® Diagnostics (respiratory panel)
  - BioGX (GI PCR reagents)
  - Apacor (ova & parasite exam reagents)
  - Diasorin (serological test reagents)

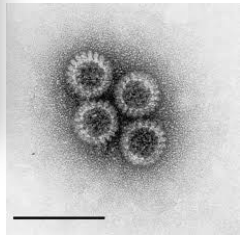
Acute Diarrhea

What do we routinely test for?

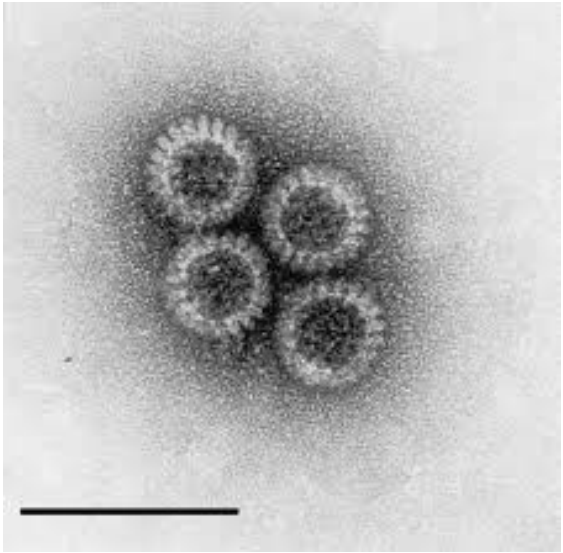
- Bacteria
- Parasites
- Viruses





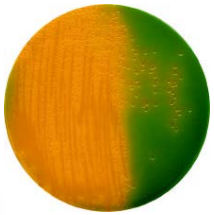


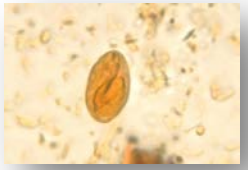
Acute Diarrhea



What is the actual prevalence

- Viruses
- Bacteria
- Parasites





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 USA[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: 727-741
[2]Mead et al. Emerg Infect Dis 1999; 5:607

Facts About GI Pathogens

1. Viruses - most prevalent; least tested[1]
   - Norovirus is #1 GI infection in the USA
   - Rotavirus declined 67% since vaccine introduction in USA

2. Bacteria - stool Cx are most common test
   - only positive 1-5% of cases[2]

3. Parasites - domestically acquired infections typically associated with defined exposure risks

[1]Guerrant et al. *Clin Infect Dis* 2001; 32:337-338
[2]Graves. *Prim Care Clin Office Pract* 2013; 40: 727-741

Community Onset/Primary Care Setting

- Viral - #1 cause of acute diarrhea
  - norovirus
- Bacterial – outbreak/cluster related
  - *Clostridium difficile* is growing
- Parasitic – sporadic, low incidence

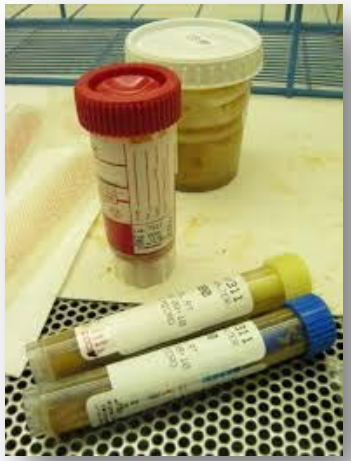
Hospitalized Patients

- HAI in acute care & ICU
  - Viral – norovirus, rotavirus
    - Emerging – sapovirus, adenovirus, astrovirus
  - Bacterial – *Clostridium difficile*
  - Parasitic – extremely rare

In Practice

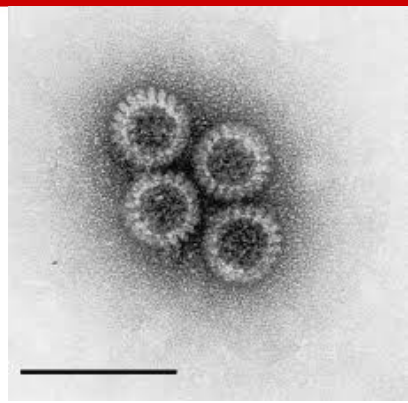
What is a common stool test ordering pattern for acute diarrhea?

- No viral tests***
- Stool Culture
- Single O&P



***(based on composite ordering pattern data from ARUP and other large academic medical centers)

Viral Testing



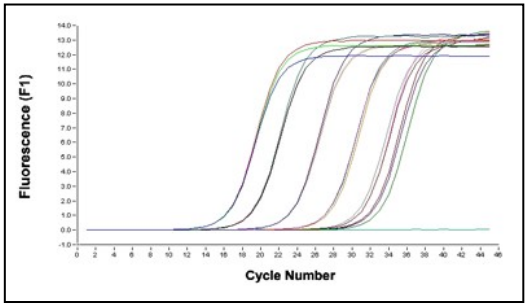
- Antigen detection EIA
  - Rotavirus & adenovirus 40/41
  - Sensitivity and specificity are good vs electron microscopy
    - Poor vs. PCR
  - **Underutilized**
- RT-PCR
  - Better sensitivity and specificity than EIA[1]
  - Norovirus: highly utilized
- No testing available for sapovirus & astrovirus

[1]Fisman *et al. J Transl Med* 2009

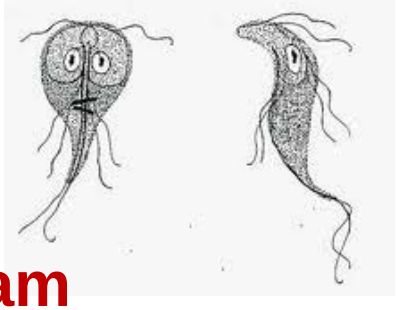
Bacterial Testing

- Culture
  - Variable sensitivity
  - Variable TAT (24-96+ hours)
  - Can become costly (multiple plates); **highly utilized**
- Antigen testing for shiga-like toxin

- *Clostridium difficile* real-time PCR
  - Multiple FDA approved methods
  - Fast, sensitive, & specific
  - Expensive, but most robust method



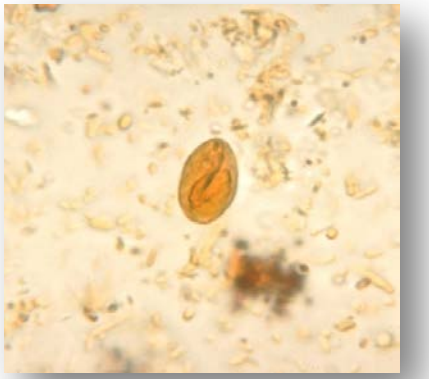
Parasite Testing



- **<u>Overutilized Ova & Parasite microscopic exam</u>**
  - Highly variable sensitivity (lab dependent)
  - Highly variable specificity (lab dependent)

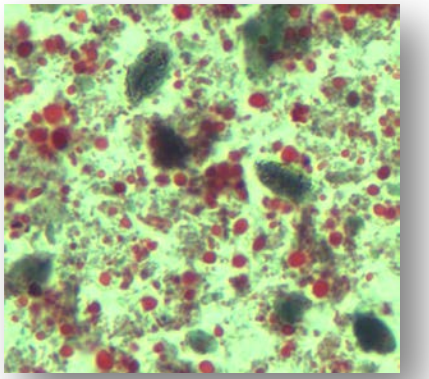
- Stool collected in fixative (preserve morphology)
  - PVA &10% Formalin
  - Single vial collection
    - Sodium Acetate Formalin (SAF)
    - Formalin free fixatives
      (*e.g.* – ParaPak SVT®, EcoFix®, TotalFix®, PROTO-FIX®, AlcorFix®, *etc*)

Ova and Parasite exam



- Concentrated wet mount preparation
  - Chemical or physical
- Permanent smeared trichrome stain

- Technologist manually exams both preparations for parasites
  - Time consuming
  - Low yield
  - Primary expense = SWAB

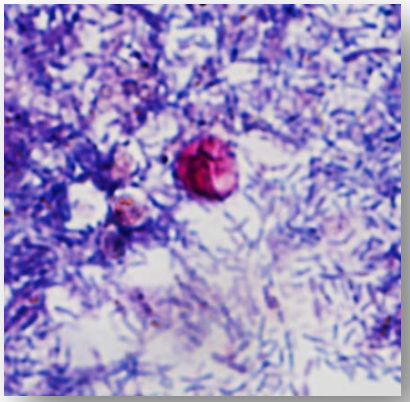


- Labs HATE this test…physicians love it!

O&P Issues

- Standard O&P does NOT readily detect:
  - *Cryptosporidium* spp.
  - *Cyclospora* spp.
  - *Cystoisospora* spp.

  → Modified acid fast +/- Modified Safranin +/- UV microscopy
  - Microsporidia → Modified trichrome



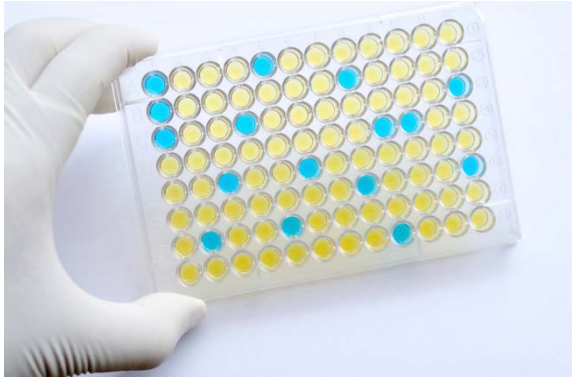
- Cannot easily differentiate *E. histolytica* from *E. dispar*
- 3+ specimens recommended/patient (span 5-7 days)
  - Rarely received

O&P Considerations

- Typically restricted to patients with high/reasonable pre-test probability
  - Immunocompromised patients
  - Pertinent exposure history (immigrants, hikers, splash parks, daycares)
  - Pertinent travel history
  - Having eaten at a commercial restaurant…
- Institutions may require prescreening for *Giardia, Cryptosporidium* first

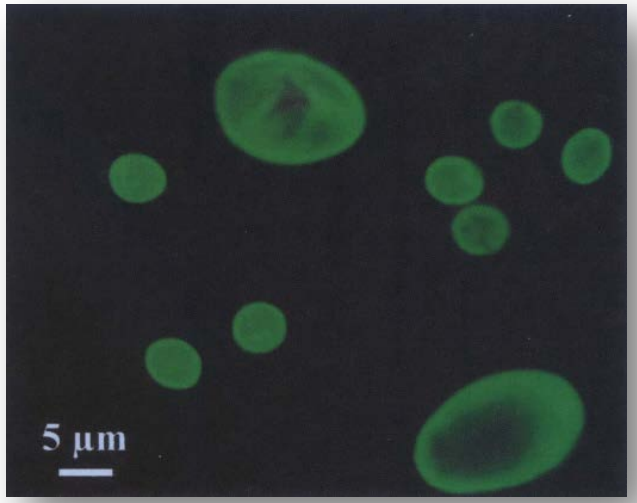
Alternative Protozoal Testing

- DFA
  - *Giardia, Cryptosporidium*
- Antigen detection ELISA or immunochromatographic assay
  - *Giardia, Cryptosporidium, E. histolytica*
  - Most assays cannot differentiate *E. histolytica/E. dispar*
- Recommended for initial screen
  - Rapid TAT, sensitive, specific



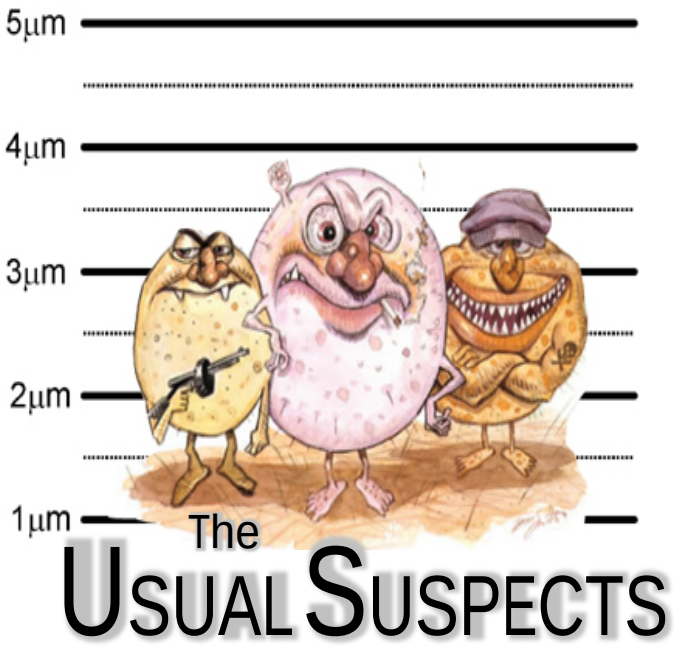
Alternative Testing Issues

- Antigen detection ELISA or immunochromatographic assay
  - *Giardia* may require multiple specimens if first specimen is negative
    - Periodic shedding of cysts
- Antigen - / stain+
  - No test is perfect
- DFA – very laborious, low throughput
- **Underutilized** when indicated in documented outbreaks[1]
  - *Cryptosporidium* SLC, 2007



[1]Polage *J Clin Micro* 2011

GI Protozoa Revisited



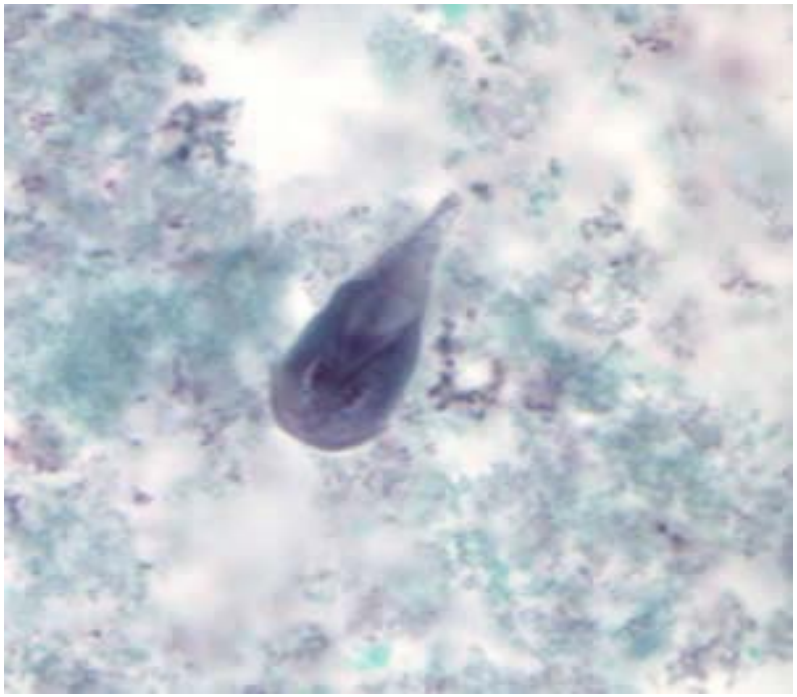
Giardia lamblia/intestinalis/duodenalis

- Binucleated, flagellated, highly pathogenic protozoa
- Endemic where there is water and beavers… and deer, dogs, cats, humans, sheep, birds…
- Fecal oral transmission including:

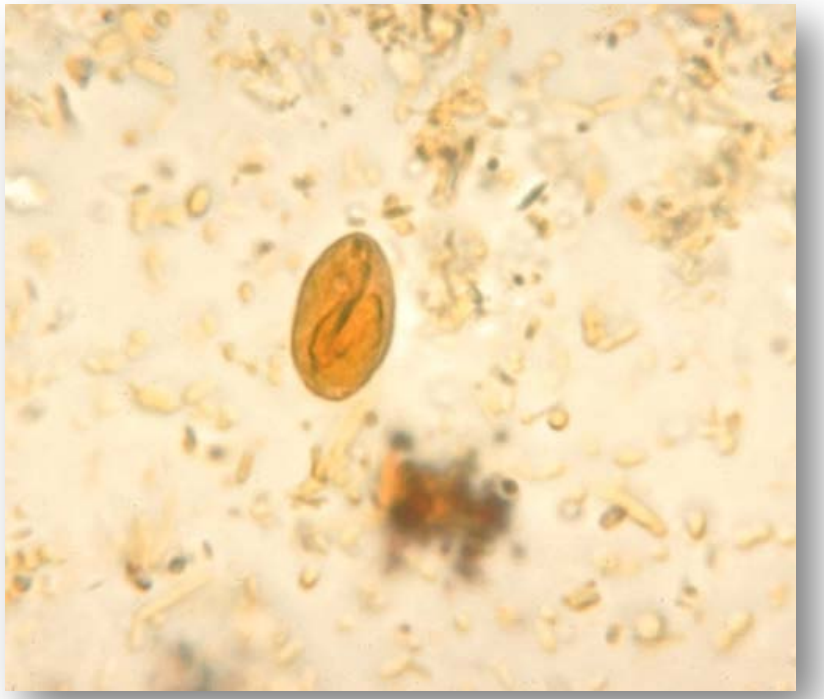




Giardia

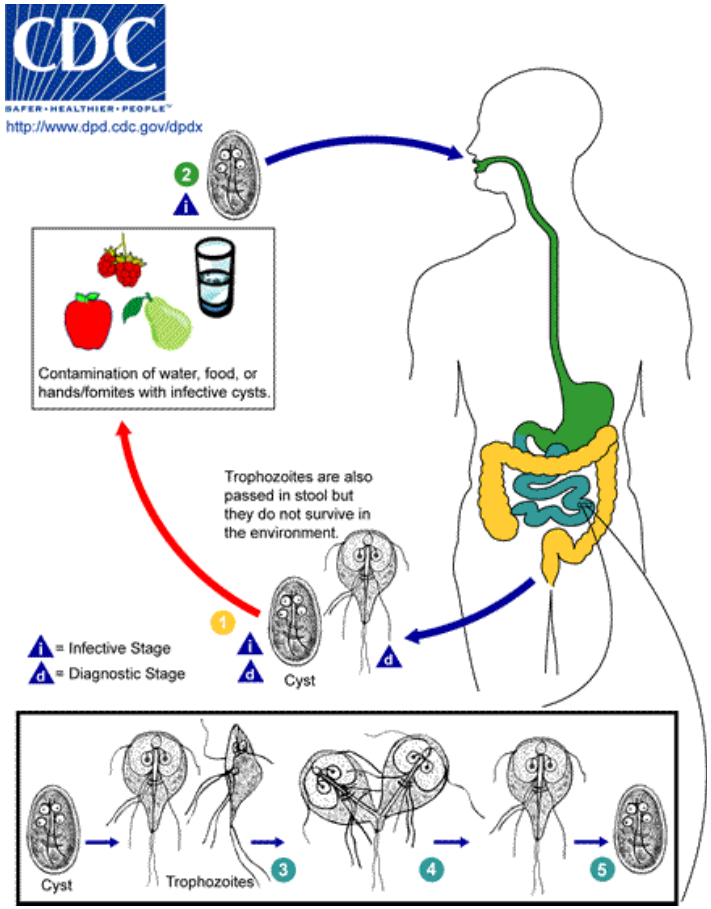


Trophozoite (10-20μm)



Cyst (10-14μm)

Giardia - symptoms



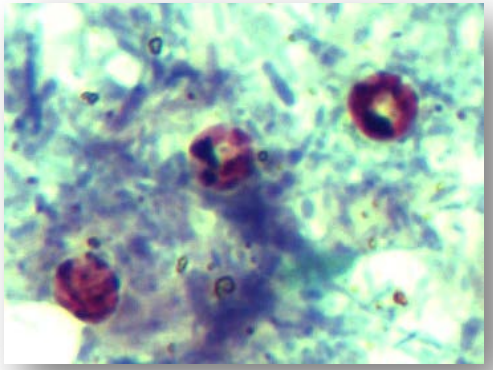
- Asymptomatic → Mild → Severe symptoms
- Diarrhea
  Malabsorption
  Abdominal pain
  Bloating
  Nausea
  Vomiting
  - 1-3 weeks
- Can become chronic

http://www.cdc.gov/dpdx/giardiasis/

Cryptosporidium spp.

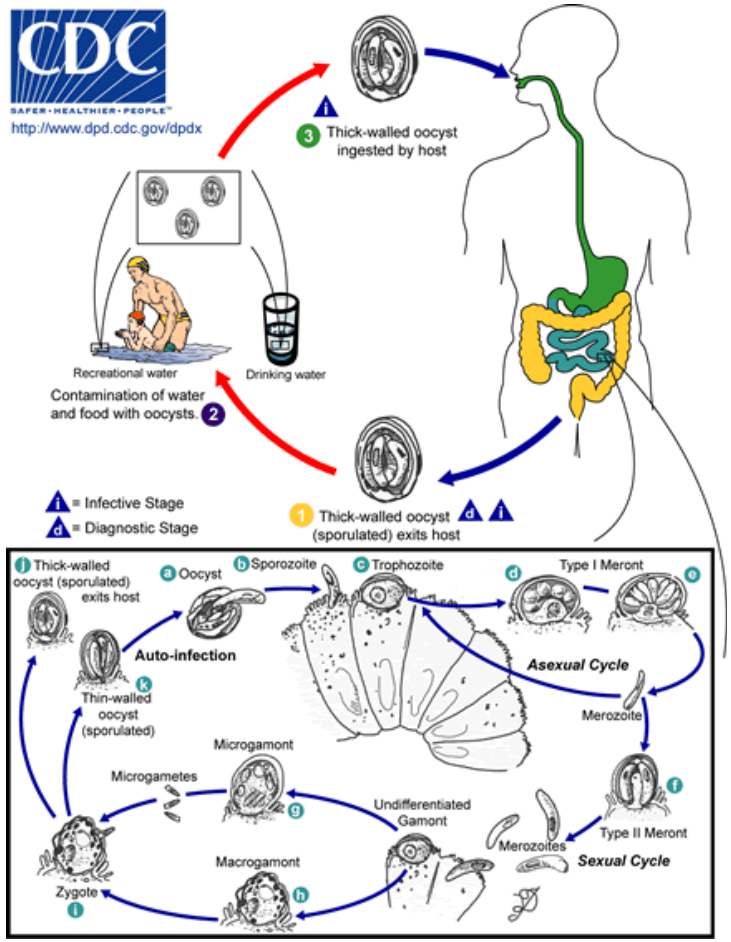
- Coccidian protozoa, stained with modified acid fast
- Transmitted fecal/oral via contaminated water
- Associated with large outbreaks
  - 2007 – SLC splash-parks/pools (5,700 cases)
  - 1993 Milwaukee PWS (403,000 cases)
  - Daycares (intermittent)

- Oocysts resistant to chlorine at normal pool concentrations



4-6 μm

Cryptosporidium spp.

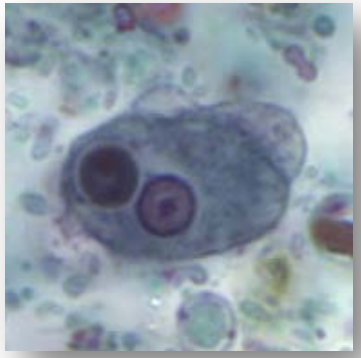


- Watery diarrhea
  (1-2 weeks); shed 2 weeks
- Cramps
  Nausea
  Dehydration
  Weight loss
  Vomiting
  Fever
  OR Asymptomatic
- Immunocompromised can shed for > month (can be chronic)
- Oocysts immediately infective when shed

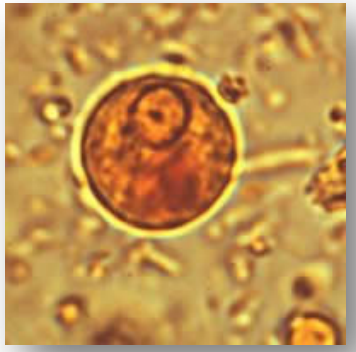
Entamoeba histolytica

- Worldwide distribution; fecal-oral
- Common in developing nations or areas of poor sanitation
- Can disseminate to liver

- Nearly indistinguishable from non-pathogenic *E. dispar* by microscopy

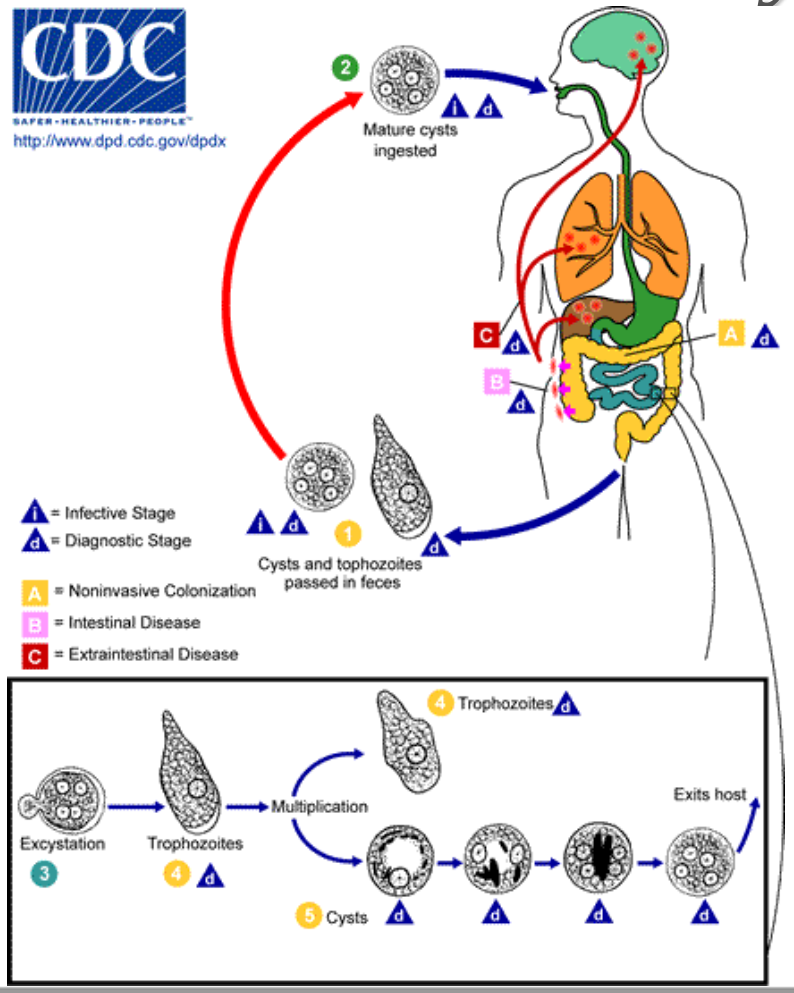


Trophozoite
15-20 μm



Cyst
10-15 μm

Entamoeba histolytica



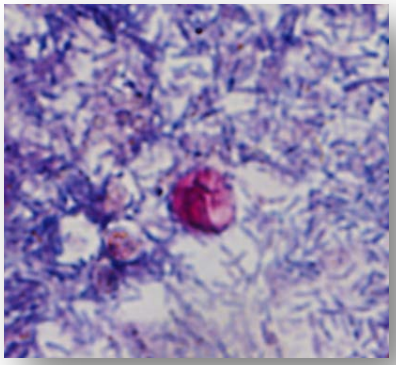
- Diarrhea in most cases with cramping OR asymptomatic
- Amoebic dysentery:
  - Fever
  - Bloody stool
  - Severe stomach pain
- Amoebic liver abscess

http://www.cdc.gov/dpdx/amebiasis/

Cyclospora cayetanensis

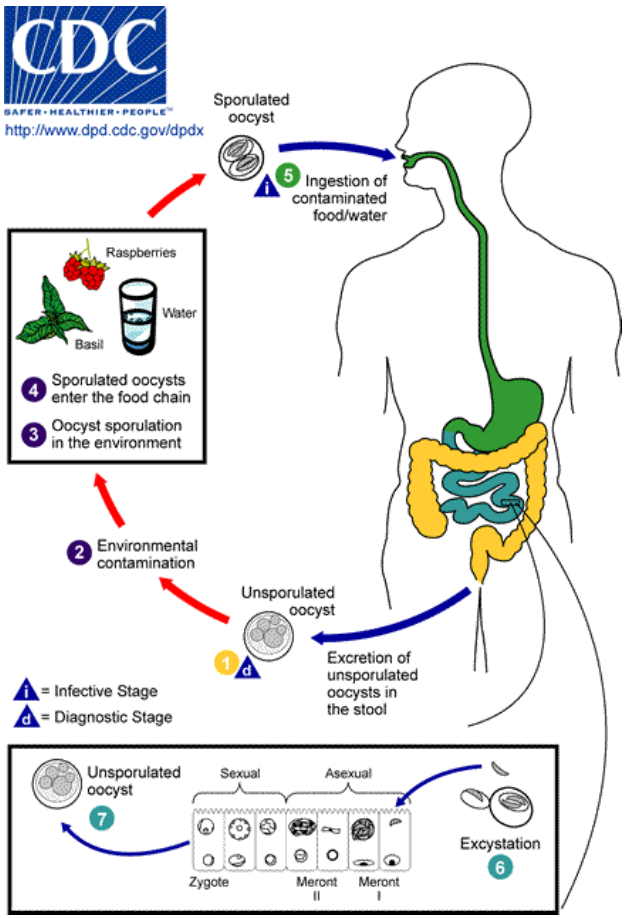
- Coccidian protozoa (similar to *Cryptosporidium*)
  - Stained with modified AF or safranin
  - Autofluorescence by UV light

- Infected humans are vector
- Tropical/subtropical regions
- 4 major recent outbreaks
  - Associated w/ bagged produce and cilantro
    - Iowa & Nebraska – July/August 2013
    - Texas – August 2013 & August 2014
    - Multistate – May – August 2015



8-10 μm

Cyclospora cayetanensis



- Watery diarrhea
  (can last months if untreated)
- Cramping
  Nausea
  Weight loss
  Loss of appetite
  Gas/bloating
  Fatigue
  OR Asymptomatic
- Vomiting & low fever (rarely)
- Oocysts not infective when shed

Protozoal Diarrhea

- Acute symptoms can mimic bacterial & viral diarrhea
- More predictive if symptoms are persistent
  - >15 days from onset
- Very predictive if chronic
  - >30 days from onset

- When to test for parasites becomes a challenge
  - Even a persistent or chronic infection starts as an acute infection

Classical GI Pathogen Testing

- Requires many different tests
- Variable sensitivity & specificity
  - Antigen especially
- Poor ordering practices or understanding of test limitations
  - Parasites especially
- Test results often not available in meaningful time
- What is the answer?

MULTIPLEX MOLECULAR DIAGNOSTICS

Rapidly evolving…the field circa November 10th, 2015

Why Multiplex Detection?

- Syndromes may be too similar to separate clinically
- Lack of standardized/differential driven ordering

  ++Cx, too many O&P's, & no viral tests

Molecular Testing Considerations

- Not appropriate in every patient
- TAT fast enough to influence care decisions?
- Will results influence clinical care?
  - Most viral/bacterial infections are self-limiting
- Positive result = stop testing = save healthcare spending?
- A test = “Excellent Patient Experience”?
  - Depends on the cost…

Molecular Testing Considerations

- Cost may be significant limiting factor
  - Who pays for this (outpatients)?
  - CPT codes released 2015
    - 87505 3-5 targets
    - 87506 6-11 targets
    - 87507 12-25 targets

- Should broad/syndromic panels be SOP?

FDA Cleared Testing Approaches

- Prodesse® Progastro™ SSCS
- BD Max™ Enteric Bacterial Panel & Enteric Parasite Panel
- Nanosphere Inc. Verigene® Enteric Pathogen test
- Luminex™ xTAG Gastrointestinal Pathogen Panel (GPP)
- Biofire Diagnostics Inc. 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

Progastro™

- Pros
  - Can replace stool culture
  - Mirrors CAP criteria for enteric pathogen detection
  - Can fit low/medium throughput volumes
  - Can be performed on frozen or Cary-Blair preserved stool

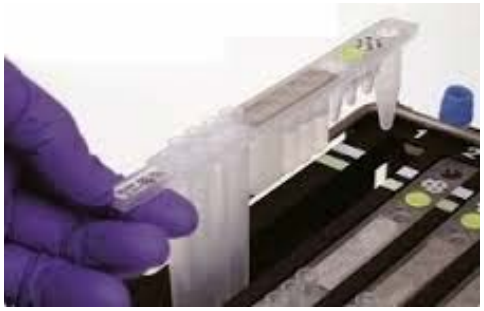
- Cons
  - Open platform, requires molecular expertise
  - Very hands on
  - Batching
  - May not allow for culture

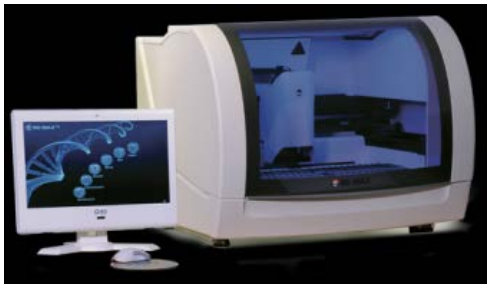
Progastro™ Performance

- 4 center study, 1244 specimens
- 100% sensitivity after molecular resolution for discrepancy
- Excellent specificity
  - Campy
    - 7 false positive: prospective
    - 5 false positive: retrospective
  - Shiga-like toxin (1 false positive)

BD Max™ Enteric Bacterial Panel

- All-in-one platform
  - Bacterial panel
- "Walkaway" PCR
- Integrated extraction and amplification





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

BD Max™

- Pros
  - Can replace stool culture
  - Mirrors CAP criteria for enteric pathogen detection
  - Can fit low/medium throughput volumes
  - Can be performed on frozen or Cary-Blair preserved stool
  - Limited hands on time

- Cons
  - Requires molecular expertise/facilities
  - Batching; semi-random access (1-24)
  - May not allow for culture

BD Max™ Performance

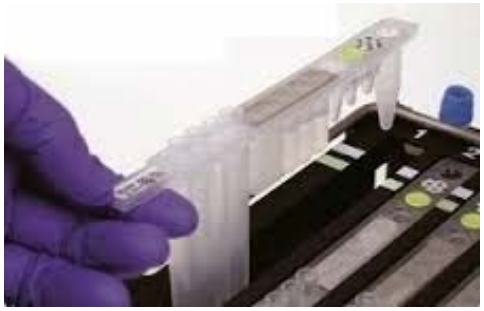
- Large multicenter evaluation (USA & Canada)
  - 4242 specimens
- Negative agreement values for all targets >98% vs Cx and/or antigen
  - Function of large study size with many negative specimens
- Positive agreement after resolution ranged 91-100%
  - Campy FN (5) & FP (31)
  - Salmonella FN (6) & FP (8)
  - Shiga-like toxin FP (8)

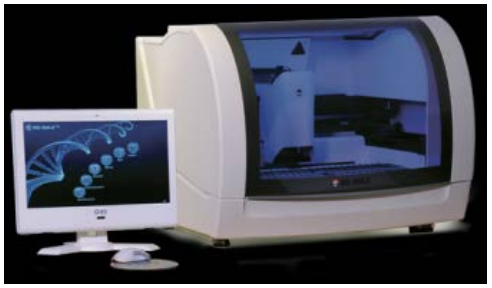
BD Max™ & Progastro™

- Replace cumbersome cultures
  - Can smaller labs handle a SmartCycler?
- Increase sensitivity for challenging organisms
  - *Campylobacter*
  - STEC

BD Max™ Enteric Parasite Panel

- Parasite panel FDA cleared (8/31/2015)
- Great opportunity to augment parasite specific testing
- No peer reviewed publications to date
- Stay tuned…





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

Verigene® Enteric Pathogens

Bacteria

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

Viruses

- Norovirus
- Rotavirus

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

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

Verigene® Enteric Pathogens

Pros

- Scalable (up to 32 analyzers/reader base unit)
- Targets the most common GI pathogens in the USA
- Limited hands on time
- Does not require molecular expertise
- Samples can be cultured
- Random access

Cons

- Targets comparatively not broad
- Modules require significant bench space
- No published performance studies to date

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

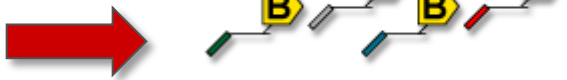
Parasites

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

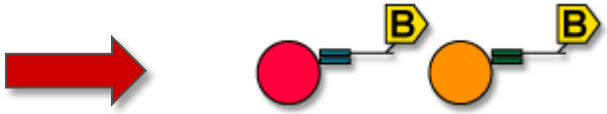
https://www.luminexcorp.com/clinical/infectious-disease/gastrointestinal-pathogen-panel/

Luminex™ xTAG GPP

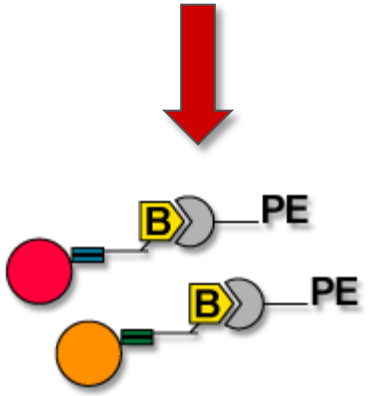
Multiplex PCR



Primer extension w/ xTAG



Tag hybridized to anti-tag coupled xMAP beads



Hybridized beads read and analyzed

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

Luminex™ xTAG GPP

Pros

- Detects wide panel of pathogens (bacterial, viral, protozoa)
- Readily detects coinfections
- Good for moderate/high volume laboratories
- Specimens may be cultured
- Can be performed on frozen or Cary-Blair preserved stool

Cons

- Requires molecular expertise/facilities
- May not allow for culture
- Long TAT
- Requires batching
- Contains *C. difficile* (Pro/Con?)

xTAG GPP Performance

- First to market, several studies
- Claas et al. 901 stools, 4 sites
- Sensitivity vs routine PCR:
  - Rotavirus (9/9), Norovirus (18/18), Giardia (22/22), *E. histolytica* (6/6)
  - Adenovirus (4/20), Cryptosporidium (21/23)
- Sensitivity vs culture:
  - *Campylobacter* (111/114)
  - Shiga-toxin producing *E. coli* (15/16)
  - *Salmonella* (62/75)
  - *Shigella* (40/40)
- Specificity all >96%

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*

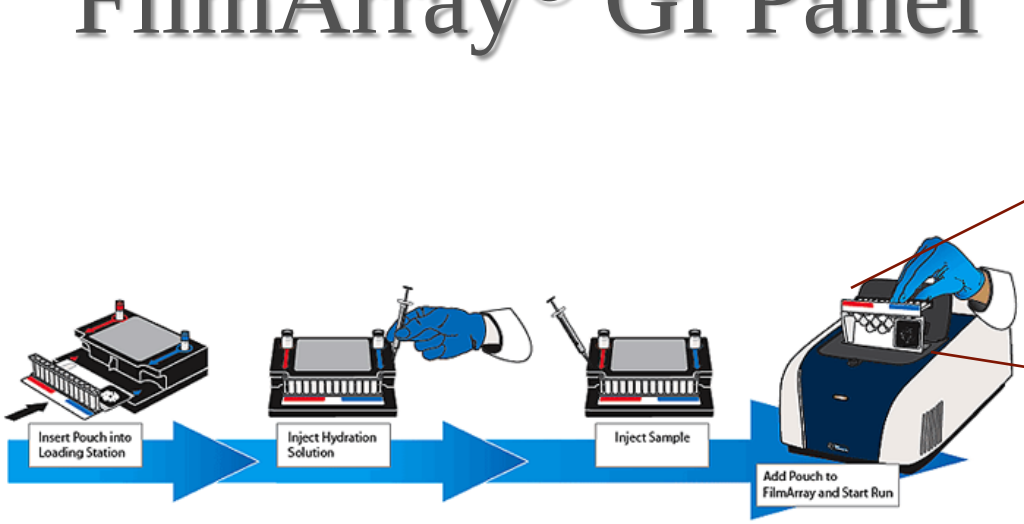
Viruses

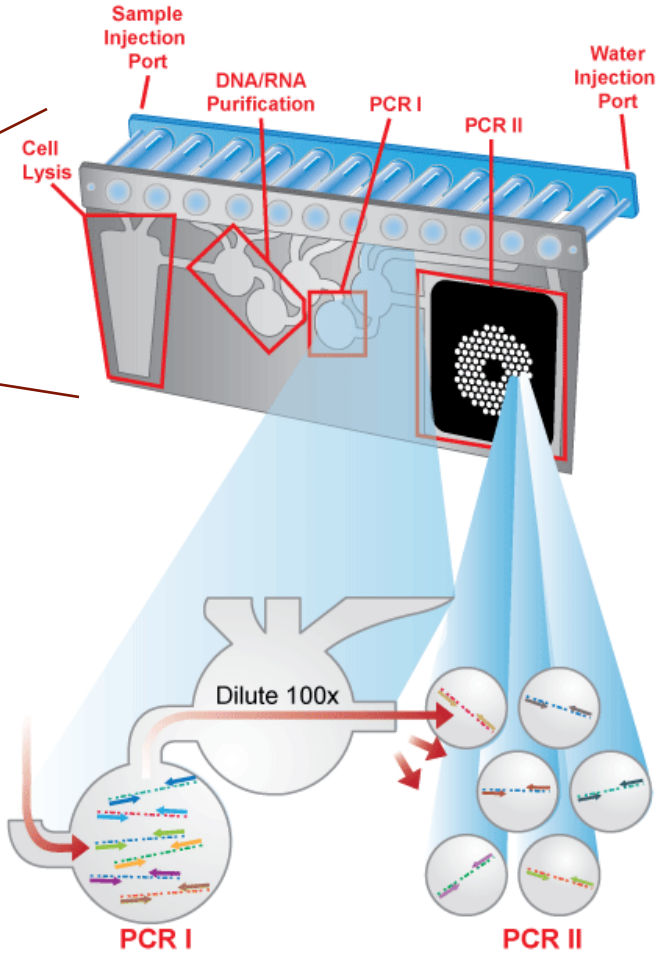
- Norovirus (GI, GII & GIV)
- Adenovirus F 40/41
- Rotavirus (A, B, C)
- Astrovirus
- Sapovirus

Parasites

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

FilmArray® GI Panel





FilmArray® GI Panel

Pros

- Detects wide panel of pathogens
- Readily detects coinfections
- Good for low volume laboratories
- Little hands on time, very simple
- Specimens can be cultured

Cons

- Requires multiple analyzers to accommodate higher volume testing
- Targets with poorly understood clinical correlation (EPEC)
- Contains *C. difficile* (Pro/Con?)
- Crossreactivity for *E. histolytica* & *E. dispar*

FilmArray® GI Panel Performance

- 4 center study, 1556 specimens[1]
- Comparators: culture or PCR
- Sensitivity all >97% except:
  - Norovirus (94.5%, 52/55), Adenovirus (95.5%, 42/44), *Shigella* (95.9%, 47/49)
- Specificity all >98%
- 262 multiple-pathogen samples identified
- *Giardia*, *Cryptosporidium, Cyclospora* 100% sensitive
  - >99.5% specific
  - *E. histolytica* none identified, 100% specific.
- 2013 *Cyclospora* outbreak "identified" at trial site using molecular[2]

[1]Buss et al. J. Clin Micro. 2015. 53(3).

[2]Buss *et al.* 2013 J. Clin Micro 51(11)

Truly Syndromic Approach

- Luminex & BioFire are very comprehensive!
- Easier for ordering purposes
  - Fewer “misses”
  - More identifications
- Great for learning your true epidemiology
  - Perceptions may not reflect realities
- Parasite testing on long-term inpatients?!?

More, Concerning Parasites?

- Clinical trials for parasites have low N=
- What is the “realistic” clinical performance versus “the old ways”?
- Will more than one sample need to be tested for periodic shedding?
- Will this solve our plights of experienced parasitologists?
- Will O&P volumes finally drop?
  - Will people forget O&P when they SHOULD order it
    - *e.g.* helminths or protozoa not targeted

ARUP's Molecular Parasitology Experience

- LDT GI Parasite PCR
  - *Giardia*
  - *Cryptosporidium*
  - *Entamoeba histolytica*
  - *Dientamoeba fragilis*
  - *Cyclospora cayetanensis*

Parasite Testing at 1 Year

- *Cyclospora* = 12
- *Cryptosporidium* = 5
- *Dientamoeba fragilis* = 3
- *Giardia* = 1
- *E. histolytica* = 1

- N = 287
  - Utah = 194 (68%)
- Positivity = 8.0%
  - 17% in first 3 months
- Inhibited = 7 (2.4%)

Case 1

26 yo female with vague gastrointestinal symptoms; mild persistent diarrhea

- O&P positive: *E. histolytica/dispar*
- *E. histolytica* IgG Serology: Positive
- *E. histolytica* Antigen EIA: Negative

- Patient from highly endemic country, definite past infections, O&P not useful, antigen test lacks sensitivity but specific for *E. histolytica*
- (After consult) PCR ordered: Positive, *E. histolytica*!

Case 2

56 yo male with recent onset, persistent diarrhea. Uncontrolled HIV → AIDS, off HAART, critically low CD4 count, critically high viral load

- O&P: Negative
- *Cryptosporidium* antigen: Negative
- Modified Acid Fast stain: Negative
- PCR: Positive, *Cryptosporidium*!

- Patient transferred from GI service to ID, started on HAART and Nitazoxinide. Followed in clinic

Case 3

- 72 y.o. Female w/pmh colon Ca.
- Several weeks of diarrhea, typically after eating, 2-3 loose stools/day (5/2015 - 6/2015)
  - Concerned for relapse of colon Ca.
- Explosive, loose, voluminous stool preceded by intense cramps
  - Yellow, no blood
- Chills and sweats, no fevers.
- From UT; no travel Hx, no Abx use
- Concern for salads she began eating regularly in recent weeks

Case 4

- 69 yo male w/pmh IBS (x26 yrs)
- 6-7 week h/o diarrhea, 3-4 watery stools/day (5/2015-6/2015)
  - Patient noted “different than IBS”
- No recent travel (lives in UT) or Abx use
- Fecal lactoferrin (+) (consistent w/IBS)
- Fecal occult blood (-)
- *C. difficile* PCR (-)

Case 4 & 5

- Cyclospora!
- Neither had specific suspicion of *Cyclospora* BUT suspicion of endemic parasites prompted Parasite PCR

What Do We Know?

- Performance for various assays generally comparable
  - All tests detect more positives than conventional testing
    - Caveat: targets are not the same between assays
- Multiple infections can and will be detected
  - FilmArray® may be most robust[1]
- Asymptomatic patients, if tested, will be positive on occasion
  - *C. difficile*, rotavirus, astrovirus, adenovirus, *Salmonella*, EPEC, ETEC, EAggEC

What Do We Know?

- Each test/system has advantages/disadvantages
  - Test may have to fit the lab
- Cultures may not go away
  - Public Health
  - AST
- Outcomes/cost effectiveness studies are needed!
  - Test utilization guidance will likely be needed

Conclusions

- Molecular GI testing will detect more positives than conventional testing
- Most commercial assays are comparable
- Parasitology stands to gain from the easy/streamline of testing
  - Increased sensitivity + convenience = better detection
- Utilization management will be critical
- Molecular GI Testing is here to stay!

Thank You