Molecular Diagnosis of Gastroenteritis/Acute Diarrhea

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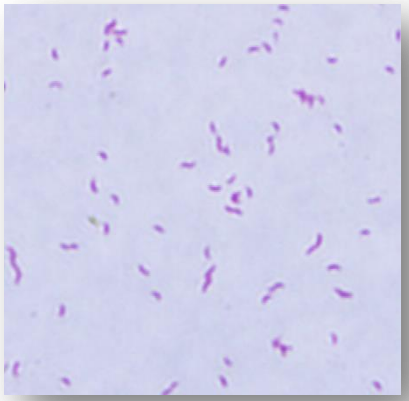
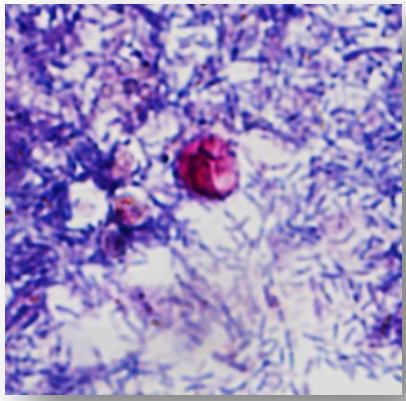




Relevant Disclosures

- Biofire Diagnostics Inc.

Objectives

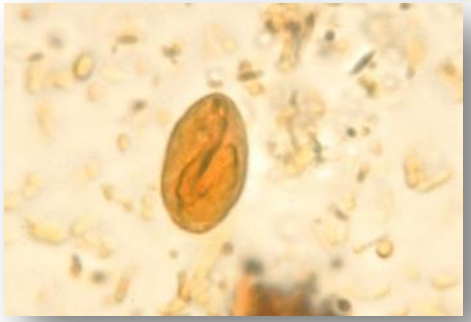
1. Discuss the epidemiology of acute diarrheal illness & approach to testing
2. Explore the new molecular panel-based methods and their potential role
3. Consider the incorporation of new diagnostic methods in clinical practice

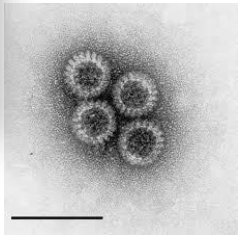
DISCLAIMER This talk is meant to be provocative and empower the audience to consider various viewpoints and approaches to the diagnosis of acute diarrhea in the setting of molecular multiplex methodologies… There is no “right answer” at this time

Acute diarrhea

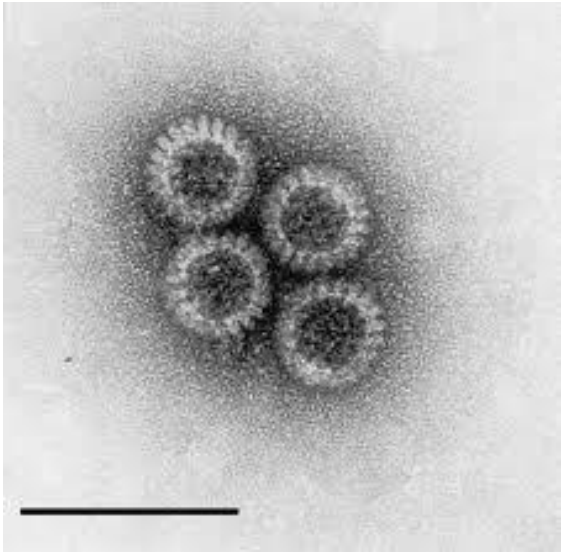
What do we routinely test for?

- Bacteria
- Parasites
- Viruses



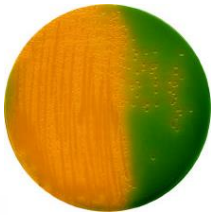


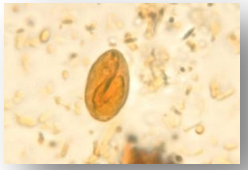
Acute diarrhea



What is the actual prevalence

- Viruses
- Bacteria
- Parasites





Acute Diarrheal illness

- Most acute GI infections are not reported or intervened medically in the USA[1]
  - Cause significant morbidity and mortality
    - More significant in developing nations
    - Prevent dehydration, provide rehydration
  - CDC estimates >350 million acute diarrheal illnesses annually[2]
  - FoodNet reports 48 million are foodborne
  - Comprehensive U.S. epidemiological reports are lacking

[1]Graves. *Prim Care Clin Office Pract* 2013; 40: 727-741
[2]Mead et al. Emerg Infect Dis 1999; 5:607

Consider some facts

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

GI pathogens in community onset/primary care setting

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

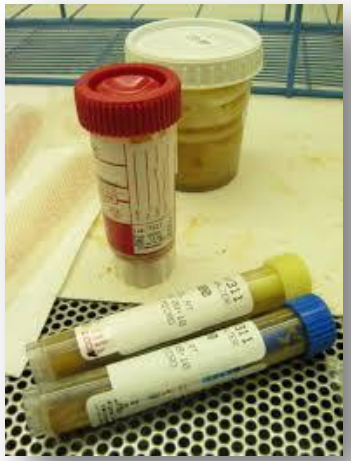
GI pathogens in hospitalized patients

- Common HAI in acute care & ICU
  - Viral – norovirus, rotavirus
    - Rare/emerging – sapovirus, adenovirus, astrovirus
  - Bacterial – *Clostridium difficile*
    - Rare – *Salmonella*
  - Parasitic – very rare

In Practice

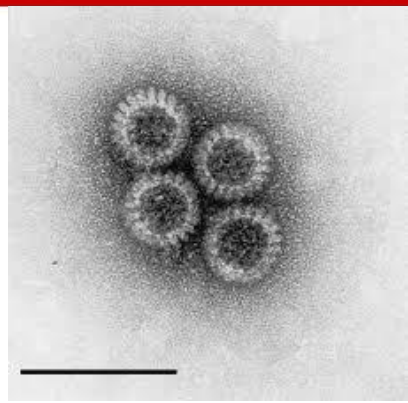
What is a common stool test ordering pattern for acute diarrhea?

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



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

Viral testing



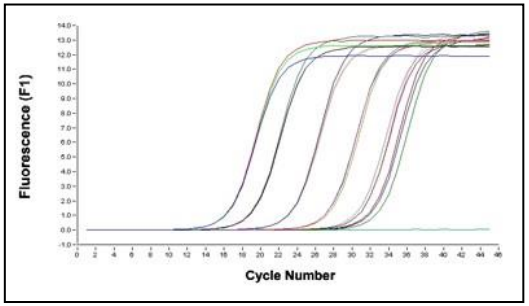
- Antigen detection EIA
  - Standard for rotavirus & adenovirus 40/41
  - Sensitivity and specificity are good vs electron microscopy
  - **Underutilized**
- PCR: available for norovirus
  - Better sensitivity and specificity than EIA[1]
  - The way of the future for others
- No testing available for sapovirus & astrovirus

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

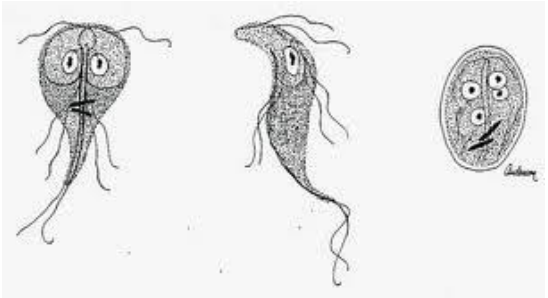
Bacterial testing

- Culture
  - Sensitivity: highly variable / excellent specificity
  - TAT: 24-96+ hours
  - Many labs use different combinations of media…can become costly

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



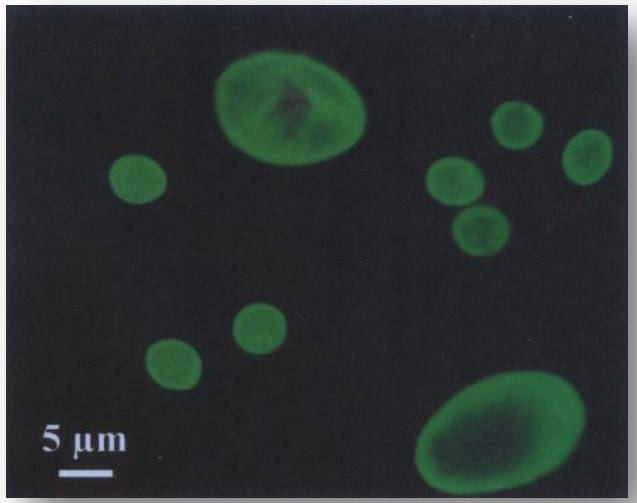
Protozoal testing



- **Overutilized O&P exams**
  - Highly variable sensitivity
- Standard O&P does NOT readily detect:
  - *Cryptosporidium* spp.
  - *Cyclospora* spp.
  - *Cystoisospora* spp.
  - Microsporidia
- 3+ specimens recommended/patient
  - Rarely received

DFA or Antigen detection

- *Giardia* and *Cryptosporidium*
  - Recommended for initial screen
  - Rapid TAT and good sensitivity and specificity
- **Underutilized** when indicated in documented outbreaks[1]



[1]Polage *J Clin Micro* 2011

The answer to ALL our diarrheal testing needs…or not?

MULTIPLEX MOLECULAR DIAGNOSTICS

Why do we need multiplex detection?

- Syndromes too similar to separate clinically
- Lack of standardized/differential driven ordering for all appropriate diarrheal agents
  - Cx, too many O&P's, & no viral tests

Molecular testing considerations

- Abundant organisms
  - less focus on analytical sensitivity
    - Are these significantly better than what we have?
- Not appropriate in every patient
  - TAT fast enough to influence care decisions?
  - Will having these results influence clinical care?
    - Most viral/bacterial infections are self-limiting & supportive care is key

Molecular testing considerations

- Cost may be <u>significant</u> limiting factor
  - Who pays for this (outpatients)?
  - How do we bill for this?
    - Per target? No CPT codes yet…

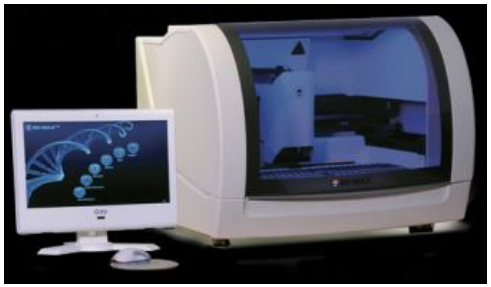
- Should large panels be standard?
  - Use mini-panels based on prevalence?
    - Viral first to cover the majority

FDA cleared testing approaches**

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

**Other products in clinical trials or submitted to FDA

Tests for bacteria only



BD Max™ enteric bacterial panel

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

Verigene® Enteric Pathogens

Bacteria

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

Viruses

- Norovirus
- Adenovirus
- Rotavirus

http://www.nanosphere.us/product/enteric-pathogens

Luminex™ xTAG GPP

Bacteria

- *Salmonella*
- *Shigella*
- *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

Parasites

- *Giardia*
- *Cryptosporidium*

FilmArray® GI Panel

Bacteria

- ETEC
- EPEC
- STEC/EHEC
- STEC 0157 serotype
- EIEC
- EAEC
- *Vibrio* spp.
- *V. cholerae*
- *Shigella* spp.
- *S. dysenteriae*
- *Salmonella* spp.
- *Campylobacter* spp.
- *Yersinia enterocolitica*
- *Clostridium difficile*
- *Aeromonas* spp.
- *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*

But do we really need ALL of this on each patient?

CONSIDER YOUR HOST

Hospitalized patients…maybe not

- Do you need to test for everything?
  - norovirus PCR
  - *C. difficile* PCR

- No FDA cleared norovirus PCR
  - LDTs – reference lab or PHL lab support
  - EIA is FDA cleared: not recommended for routine testing (50% sensitivity) → reflex to PCR

Return traveler…maybe so

- Chronic diarrhea
  - Parasites likely depending on exposures; therapy likely indicated[1]
  - Also consider O&P's for helminths or other protozoa
    - *e.g. Balantidium coli, Dientamoeba fragilis, Cystoisospora, Cyclospora*
    - Molecular doesn't cover everything!
- Bacterial causes are highly variable
  - *Vibrio, Campylobacter, Shigella dysenteriae, Salmonella typhi,* ETEC, EAggEC
    - If ongoing symptoms, treatment may be considered
    - Empiric therapy for ETEC is standard[1]

[1]Guerrant. *Clin Infect Dis* 2001

Immunocompromised hosts…maybe so

- Pathogens not consistent in each group
- Patients typically receive extensive work-up
  - ➢ More likely to have interventions in care[1]

  – HIV+
  – Primary immunodeficiency

  } Viruses, *C. diff, Campy, Shigella, Salmonella, E. coli, Giardia, Crypto,* Microsporidia

  – Solid organ transplant
  – Stem cell transplant

  } Most diarrhea is NOT infectious
  Viruses (noro), *C. diff*
  Microsporidia

[1]Guerrant. *Clin Infect Dis* 2001

Healthy pediatric patients…maybe so

- Viral is most common[1]
  - Norovirus rising, rotavirus declining (in vaccinated countries)
  - Adenovirus, sapovirus, astrovirus also significant
- *C. difficile* on the rise in non-infants[2]
- Exposures that adults don't typically have
  - excessive fecal-oral exposures (daycares)[3]
- Likely actionable changes to management based on severity[3,4]
  - Guidelines do not recommend treating all pediatric gastrointestinal infections with antibiotics

[1]Anderson. *Expert Rev Anti Infect Ther* 2010
[2]Sammons and Toltzis. *Curr Opin Pediatr* 2013
[3]Guerrant. *Clin Infect Dis* 2001 *Opin Gastroentorol* 2010
[4]Szajewska and Dziechiarz. *Curr*

Primary care/community…maybe not

- Lower potential for intervention in care
  - Most will not require treatment
- Long TAT due to transport to central lab/reference lab
  - Utility is lost after several days (see Cx & O&P)[1]
    - Viral is #1…symptoms will likely resolve
    - Resolved symptoms but outstanding bill
- Should you even test?

[1]Guerrant. *Clin Infect Dis* 2001

Complicated outpatient…maybe so

- Advanced age >65
  - *Salmonella & Campylobacter* likely treated[1]
- Comorbidities
  - *e.g.* Heart disease, aortic graft, diabetes, chronic kidney disease
- Dysentery or blood in stool
  - Knowing STEC can be critical (no Abx)
    vs invasive *Salmonella/Shigella/Campy* (+/- Abx)
- Duration of illness
  - >14 days = persistent…more likely parasitic or Microsporidia
    - Treatment often indicated

[1]Guerrant. *Clin Infect Dis* 2001

Outpatient use & outbreak identification?

- Observed cluster/suspected outbreak of unknown/unpredictable origin
  - *Cyclospora* with imported produce (2013 & 2014)[1]
  - *Campylobacter* in Alaskan snow peas (2011)[2]
  - STEC in cookie dough (2009)[3]
  - Sapovirus (2002-2009) [4]
- Once N=? cases confirmed, perform directed testing with classical methods first?
  - Cost containment
  - Are the previous methods good enough here?

[1]CDC *MMWR* 2013
[2]Gardner et al. *Clin Infect Dis* 2011
[3]Neil et al. *Clin Infect Dis* 2012
[4]Lee *et al. EID* 2012

Implementation

- Assays are expensive; cost effective implementation often involves cessation of Cx
  - Will the lab maintain selective media for specific isolation of pathogens?
  - No Cx = No AST capabilities when needed

- But…smaller laboratories may replace Cx completely
  - FDA cleared molecular assay performed according to package insert is better than a poorly performed classical test
    - *Campylobacter* Cx – variability nationwide: Cx time, transport conditions, atmosphere, plated media[1,2]
    - …performing O&P for *Cryptosporidium, Cyclospora*

[1]Hurd et al.. *Clin infect Dis* 2012
2M'ikanatha et al. *EID* 2012

Public Health Concerns

- Culture independent assays = ↑ positives
  - Are PHLs ready?
  - Who does the culture (who pays)?
    - What if we have no isolates…
  - Can you EVEN culture the specimens?
    - Luminex – No
    - BD Max & Prodesse – Maybe
    - Biofire, Verigene - Yes

Timeline of an STEC outbreak investigation

Day 0: STEC isolate identified at (3-10 days post ingestion)

Day 1-7: PHL receives strain and performs typing & fingerprinting

Day 8-12: PHL reports case with appropriate outbreak information

Total time: 2-3 weeks from exposure…WITH an isolate

What happens without isolates…

Bigger outbreaks? Incomplete reporting?
Underestimates of outbreak sizes?

Alternatively, panel tests may identify outbreaks better upfront…and direct culturing efforts

Conversations with PHL are imperative BEFORE implementation

Coming full circle

- Panel-based GI testing is here to stay
- Not all patients likely need this testing
- Consider:
  - Cost
  - Influence (if any) on care decisions
  - Adequacy of prior methods
  - Pre-test probability of a pathogen
- Cost-effectiveness studies are needed to guide us!
- Talk to your PHL sooner than later

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The University of Utah
Department of Pathology