Fecal Me This, Fecal Me That – Where Are We Now With Giardia and Cryptosporidium Diagnostics?

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Objectives

• Discuss giardiasis and cryptosporidiosis disease and epidemiology

• Identify the strengths and weaknesses of gold standard methods

• Describe current diagnostic methods for detecting *Giardia* and *Cryptosporidium* in clinical specimens

• Review the impact these advanced methodologies have on improving diagnosis.
WHO facts

- Around 1.7 billion cases of diarrheal disease occur globally every year

- Diarrheal disease is the second leading cause of death in children under 5 years old
  - Approx. 760,000 deaths/year

- Diarrhea is a leading cause of malnutrition in children under five years old

- Most cases of diarrheal disease can be prevented with safe drinking-water, adequate sanitation and hygiene
Diarrheal disease

• Acute diarrhea
  • Symptoms <14 days

• Persistent diarrhea
  • Symptoms 14 – 30 days

• Chronic diarrhea
  • Symptoms >30 days
Approach to evaluation & management of infectious diarrhea

Initial assessment
- Dehydration
- Duration (>1 d)
- Inflammation (fever, blood in stool, tenesmus)

Provide symptomatic treatment
- Rehydration
- Treatment of symptoms
  (if necessary, consider bismuth subsalicylate or loperamide if diarrhea is not inflammatory or bloody)

Stratify subsequent management according to clinical and epidemiological features
- Clinical clues: Bloody diarrhea, abdominal pain, dysentery, wasting, fecal inflammation
- Epidemiological clues: Food, antibiotics, sexual activity, travel, day-care attendance, outbreaks, season

Adapted from Thielman & Guerrant 2004 NEJM 3510:38-47
Approach to evaluation & management of infectious diarrhea - continued

**Obtain fecal specimen for analysis if severe, bloody, inflammatory, or persistent diarrhea or if outbreak suspected**

### Community-acquired or traveler’s diarrhea
- Culture or test for:
  - Salmonella, shigella, campylobacter
  - *E. coli* O157:H7 + shiga-like toxin (if bloody diarrhea or HUS)
  - *C. difficile* toxins A and B (if recent antibiotics)

### Nosocomial diarrhea (onset >3 d after hospitalization)
- Test for:
  - *C. difficile* toxins A/B
  - Salmonella, shigella, campylobacter (if outbreak or if patient is >65 y with coexisting conditions, or if systemic enteric infection suspected)
  - Shiga toxin-producing *E. coli*

### Persistent diarrhea (>7 days)
- Consider protozoa:
  - Giardia, Cryptosporidium, Cyclospora, Isospora belli + screening for inflammation

### If patient is immunocompromised (especially if HIV+)
- Test for:
  - Microsporidia, Mycobacterium avium complex, Cytomegalovirus

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Adapted from Thielman & Guerrant 2004 NEJM 3510:38-47
Giardia background

- First described in 1681 by Van Leeuwenhoek (own stool)
- Most common intestinal human parasite in the U.S.
- Hospitalizations due to giardiasis is estimated to cost ~$34 million/year
Giardia epidemiology

- CDC estimates approximately 1.2 million cases occur annually
- Fecal-oral and person-person transmission
- Ingestion of infective cysts
  - Moderately tolerant to chlorine
  - Shedding can occur for months, $10^8$-$10^9$ cysts/day
- Primarily associated with ingestion of human waste
  - Drinking contaminated water from lakes, rivers, swimming pools
    - Backpackers, swimmers
  - Child-care facilities (children & caregivers)
  - Occupational exposure
  - Sexual practices with fecal contact
Giardiasis

- Symptoms last for 1-2 weeks and generally involve the following:
  - Diarrhea
  - Gas
  - Greasy stools, tendency to float
  - Stomach/abdominal cramps
  - Nausea/vomiting
  - Dehydration
  - Weight loss, malabsorption of fat, vitamins A & B12
  - Asymptomatic
Cryptosporidium background

- First human case of cryptosporidiosis described in 1976
  - 1980’s: Identified as cause of diarrhea in AIDS patients
  - 1993: Largest outbreak documented in Milwaukee
    - 403,000 people with diarrheal illness
- Hospitalizations due to cryptosporidiosis are thought to cost ~$45.8 million/year
Cryptosporidium epidemiology

- CDC estimates approximately 748,000 cases occur annually but <2% are actually reported

- Fecal-oral and person-to-person transmission
  - Occurs worldwide, except Antarctica

- Ingestion of infective cysts
  - Stable in environment up to 6 months in moist environments
  - Refractory to chlorination and bleach

- Primarily associated with contaminated water (drinking sources & recreational water) day care center and nosocomial transmissions
  - Less commonly linked in foodborne infections

Huang & White 2006 Gastroenterol Clin N Am 35:291-314
Cryptosporidiosis

- Onset 2-10 days post exposure
- Symptoms last for 1-2 weeks and generally involve the following:
  - Profuse, water, nonbloody diarrhea
  - Stomach cramps/pain
  - Dehydration
  - Nausea
  - Vomiting
  - Fever
  - Weight loss
  - Asymptomatic

[cdc.gov, bbc.com]
Crypto & HIV

- Prior to effective antiretroviral therapies, cryptosporidiosis diagnosed primarily in AIDS patients
- Symptoms can vary from a mild, self-limited disease to chronic diarrheal illness and sometimes fatal
  - Severity often associated with CD4 counts
  - Respiratory tract involvement
  - Biliary involvement (e.g. sclerosing cholangitis, pancreatitis)

Huang & White 2006 Gastroenterol Clin N Am 35:291-314
Disease seasonality

- Number of case reports by date of symptom onset
  - Giardia: date unknown for 14,876 of 31,981 cases
  - Crypto: date unknown for 4,740 of 12,581 cases

- Data from 2011-2012

Painter et al 2015 MMWR 64(SS03):1-25
Diagnostics: gold standard methods for *Giardia* and Crypto

- Ova & parasite (O&P) exam
  - Concentration techniques to improve sensitivity
  - Permanent stains (iron hematoxylin and trichrome) aid in parasite morphology identification
  - Wet mounts to observe motility
- Modified acid fast stain
  - Provides contrast of MAF-positive parasites (red) from fecal material in background (blue)
Conventional method advantages

• Reagent costs are relatively inexpensive
• Standardized procedures across labs
• Detects numerous parasitic protozoa and helminth ova
• Detects intact parasites versus remnant nucleic acids
Conventional method disadvantages

- O&P exams aren’t all-inclusive and additional tests (e.g. MAF stain) may be needed for some pathogens
  - Conventional staining is only ~50% as sensitive as direct fluorescence assay (DFA) for these two pathogens*

- VERY time-consuming; labor intensiveness increases overall costs

- Highly skilled technical expertise required for reading slides

- Reliant on proper collection and preservation

- Usually unable to differentiate *E. histolytica* and *E. dispar*

*Alles et al 1995 JCM 33:1632-1634*
Physician-based surveys: Use of parasite tests

- <25% of physicians surveyed in Connecticut ordered appropriate test for *Cryptosporidium* despite suspicion of cryptosporidiosis
  - Morin *et al* 1997 Arch Intern Med 157:1017-1022

- 76% of respondents assumed *Cryptosporidium* testing was included with O&P exams

- Additionally, physicians ordered parasite-based tests despite bacterial- and/or viral-like presentations
Parasite test ordering at a national referral lab

- Of 170,671 episodes analyzed from 1997-2006, majority were tested by O&P only compared to enzyme immunoassay (EIA) only or EIA + O&P

- Significantly more *Giardia* & Crypto were detected when tested by EIA when compared to O&P

Adapted from Polage et al 2011 JCM 49:591-596
Things to consider for “non-conventional” enteric parasite diagnostics…

- Would need to maintain the “ova” in O&P
  - Helminth eggs are not detected in immunoassays or PCR panels

- Does it have our institution’s most common pathogens?
  - If no, does that even matter??

UCLA positives

- B. hominis
- Giardia
- D. fragilis
- Cryptosporidium
- E. histolytica

Case Control Study (Netherlands*)

*Bruijnesteijn van Coppenraet et al., CMI 2015
Overview of diagnostic methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Tests for:</th>
<th>Turn-around time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stool culture</td>
<td>Bacteria</td>
<td>2-3 days</td>
</tr>
<tr>
<td>O&amp;P exam</td>
<td>Parasites</td>
<td>Several days – samples must be collected over multiple days</td>
</tr>
<tr>
<td>Rapid tests</td>
<td>One or two pathogens</td>
<td>10-30 min</td>
</tr>
<tr>
<td>ELISA</td>
<td>One or two antigen/antibodies</td>
<td>6-24 hrs</td>
</tr>
<tr>
<td>Real-time PCR</td>
<td>Multiple pathogens</td>
<td>&lt;5 hours</td>
</tr>
</tbody>
</table>

Adapted from Luminex brochure
## Overview: Immunoassays

<table>
<thead>
<tr>
<th>Test</th>
<th>Manufacturer</th>
<th>Time to result</th>
<th>Assay type</th>
<th>IVD?</th>
<th>Format</th>
</tr>
</thead>
<tbody>
<tr>
<td>ImmunoCard STAT! Crypto/Giardia</td>
<td>Meridian</td>
<td>10 min</td>
<td>Chromatographic</td>
<td>Yes</td>
<td>Single</td>
</tr>
<tr>
<td>MERIFLUOR Cryptosporidium/Giardia</td>
<td>Meridian</td>
<td>30 min</td>
<td>DFA</td>
<td>Yes</td>
<td>Single</td>
</tr>
<tr>
<td>GIARDIA/CRYPTOSPORIDUM QUIK CHEK</td>
<td>Tech Lab (via Alere)</td>
<td>30 min</td>
<td>Chromatographic</td>
<td>Yes</td>
<td>Single</td>
</tr>
<tr>
<td>GIARDIA/CRYPTOSPORIDUM CHEK</td>
<td>Tech Lab (via Alere)</td>
<td>&lt;2 hr</td>
<td>ELISA</td>
<td>Yes</td>
<td>96-well</td>
</tr>
<tr>
<td>Xpect Giardia/Crypto</td>
<td>ThermoScientific</td>
<td>15 min</td>
<td>Chromatographic</td>
<td>Yes</td>
<td>Single</td>
</tr>
<tr>
<td>ProSpecT Giardia/Cryptosporidium</td>
<td>ThermoScientific</td>
<td>&lt;2 hr</td>
<td>ELISA</td>
<td>Yes</td>
<td>96-well</td>
</tr>
<tr>
<td>Crypto Giardia Test Kit</td>
<td>Cardinal Health</td>
<td>30 min</td>
<td>Chromatographic</td>
<td>Yes</td>
<td>Single</td>
</tr>
<tr>
<td>Crypto-Giardia Duo-Strip</td>
<td>Corisbio</td>
<td>15 min</td>
<td>Chromatographic</td>
<td>No</td>
<td>Single</td>
</tr>
</tbody>
</table>
ImmunoCard STAT! Crypto/Giardia

- **Specimen type:**
  - 10% formalin, SAF, MIF
  - Cary-Blair, C&S, or Stuart’s
  - Fresh (diluted 1:4)

- **Time to results:** 10 min

- **Performance:**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giardia</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Crypto</td>
<td>97.3%</td>
<td>100%</td>
</tr>
</tbody>
</table>
MERIFLUOR Cryptosporidium/Giardia

- Specimen type:
  - 10% formalin, SAF
  - ExoFix

- Time to results: 10 min

- Performance:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giardia</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Crypto</td>
<td>97%</td>
<td>94%</td>
</tr>
</tbody>
</table>
GIARDIA/CRYPTOSPORIDIUM QUIK CHEK & CHEK Assays

- **Specimen type:**
  - Routine O&P specimens (no SAF for QUIK CHEK)
  - Fresh/frozen unpreserved (QUIK CHEK only)

- **Time to results:**
  - 30 min (QUIK CHECK)
  - <2 hr (CHEK)

- **Performance:**

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>97.6-100%</td>
<td>99.8-100%</td>
</tr>
</tbody>
</table>
Crypto Giardia Test Kit

- **Specimen type:**
  - Acceptable transport types not found on site

- **Time to results:** 30 min

- **Performance:**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giardia</td>
<td>100%</td>
<td>N/A</td>
</tr>
<tr>
<td>Crypto</td>
<td>100%</td>
<td>N/A</td>
</tr>
</tbody>
</table>

- **Note:** detects antigens specific for *G. lamblia* & *C. parvum*
ProSpecT Giardia/Cryptosporidium

• Specimen type:
  • Fresh/frozen unpreserved
  • 10% formalin, SAF, MF
  • Cary-Blair
  • Rectal swab/diapers

• Time to results: <2 hr

• Performance:

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>98.9%</td>
<td>99.6%</td>
</tr>
</tbody>
</table>
Xpect Giardia/Crypto

- Specimen type: stool
  - Fresh/frozen
  - 10% formalin
  - SAF
  - Cary-Blair or C&S

- Time to results: 15 min

- Performance:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giardia</td>
<td>95.8%</td>
<td>98.5%</td>
</tr>
<tr>
<td>Crypto</td>
<td>96.4%</td>
<td>98.5%</td>
</tr>
</tbody>
</table>
Crypto-Giardia Duo-Strip

- Specimen type:
  - Cannot be treated with formaldehyde or derivatives
- Time to results: 15 min
- Performance:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giardia</td>
<td>100%</td>
<td>97.3%</td>
</tr>
<tr>
<td>Crypto</td>
<td>95.7%</td>
<td>100%</td>
</tr>
</tbody>
</table>
General testing considerations

- Single-test format
  - On-demand testing
  - Lower throughput

- Multi-test format
  - Batch-testing
  - Higher throughput
  - May need extra equipment
    - Spectrophotometer, thermocycler
Example lab algorithm

Test requested

G/C EIA
- Adult (≥15 y) ± History
  - G/C EIA only
- Child (≤ 14 y) ± History
  - G/C EIA + stained smear for D. frag

O&P
- Adult (≥15 y) No history
  - G/C EIA only
- Child (≤14 y) No history
  - G/C EIA + stained smear for D. frag
- Any age + history
  - G/C EIA + stained smear + concentrate

Adapted from Church et al 2005 Arch Pathol Lab Med 129:754-759
Impact of algorithm implementation

- Reduction in full O&P examination to 1/3rd of what was previously done
- Overall decrease in total number of orders for enteric parasite stool tests – average decrease of 25.3%
- More cases overall of *Giardia* and Crypto were found by performing EIA screen
- >90% of results using EIA screen reported in under 24 hrs compared to most stool O&P results reported within 72 hrs
- Estimated costs if lab had continued to offer O&P without EIA screen would have been 21% more than current total cost of current algorithm
  - Cost of EIA screen ($17.29) remained higher than that of O&P ($14.74)

Adapted from Church *et al* 2005 Arch Pathol Lab Med 129:754-759
Improved pathogen detection with molecular testing

• 397 patient specimens tested for common protozoan pathogens by microscopy versus real-time PCR

• Targets: *G. lamblia*, *Cryptosporidium* spp., *E. histolytica*, and *D. fragilis*

<table>
<thead>
<tr>
<th>Method</th>
<th>% positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>24.2</td>
</tr>
<tr>
<td>Real-time PCR</td>
<td>38.3</td>
</tr>
</tbody>
</table>

de Boer *et al*, JCM, 2010
## Overview: commercial molecular assays

<table>
<thead>
<tr>
<th>Aspect</th>
<th>BD MAX*</th>
<th>FilmArray</th>
<th>Luminex xTAG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Targets</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria/toxins</td>
<td>4</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Parasites</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Viruses</td>
<td>0</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td><strong>Extraction</strong></td>
<td>All-in-one</td>
<td>All-in-one</td>
<td>Off-board</td>
</tr>
<tr>
<td>Throughput</td>
<td>High</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Time to result</td>
<td>~3 hr</td>
<td>1 hr</td>
<td>&lt;5 hrs</td>
</tr>
<tr>
<td>Ease of use</td>
<td>Easy</td>
<td>Easy</td>
<td>Moderately difficult</td>
</tr>
<tr>
<td>IVD?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Two panels: Enteric Bacterial Panel and Enteric Parasite Panel*
## Test menu on molecular platforms

<table>
<thead>
<tr>
<th></th>
<th><strong>BD MAX</strong></th>
<th><strong>FilmArray</strong></th>
<th><strong>Luminex xTAG</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tests</strong></td>
<td>Enteric bacterial, Enteric parasite, GBS, <em>C. difficile</em>, MRSA, MRSA XT, StaphSR, Vaginitis, CT/GC/TV, CRE</td>
<td>GI pathogens, Resp. pathogens, Blood culture ID, Meningitis/encephalitis</td>
<td>Enteric pathogens, Respiratory viruses, Multiple ASRs for individual targets (bacterial, parasitic, and viral pathogens)</td>
</tr>
<tr>
<td><strong>LDT-capable</strong></td>
<td>LDT-capable</td>
<td>No LDT</td>
<td>LDT-capable</td>
</tr>
</tbody>
</table>
BD MAX Enteric Parasite Panel

- Specimen type:
  - Unpreserved
  - 10% formalin

- Time to results: ~3 hr

- Performance:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giardia</td>
<td>95%</td>
<td>95%</td>
</tr>
<tr>
<td>Crypto</td>
<td>90.3-100%</td>
<td>99.8-100%</td>
</tr>
</tbody>
</table>

- Note: detects *G. lamblia*, *C. hominis* and *C. parvum*
FilmArray GI Panel

- Specimen type:
  - Cary-Blair

- Time to results: 1 hr

- Performance:

<table>
<thead>
<tr>
<th>Target</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasites</td>
<td>94.5-100%</td>
<td>99.5-100%</td>
</tr>
</tbody>
</table>
Luminex xTAG GPP

• Specimen type:
  • Fresh and frozen
  • Cary-Blair

• Time to results: <5 hr

• Performance:

<table>
<thead>
<tr>
<th>Target</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giardia</td>
<td>100%</td>
<td>96.9-98.9%</td>
</tr>
<tr>
<td>Crypto</td>
<td>91.3-91.7%</td>
<td>100%</td>
</tr>
</tbody>
</table>
General testing considerations

- Molecular assays
- Targets desired
- Cleared specimen types
- Performance of targets; all FDA-cleared?
- Specimen capacity
- Expanded test menu
- Footprint of instrument(s)
- Conducive for resource-limited setting?
Best practices for stool parasite testing

• Provide guidance on appropriate testing methods for particular pathogens

• Educate physicians on limitations of conventional “gold standard” methods

• Limit testing, if possible, to appropriate patients with appropriate clinical presentations, such as:
  • Inpatient <3 days
  • Acute diarrhea >7 days (most patients)
Conclusions

• Clinical labs are rapidly adopting immunoassays and molecular diagnostics in lieu of conventional staining and microscopy procedures to improve sensitivity

• Labs may wish to consider algorithmic-based workflows to reduce unnecessary use of O&P exams

• Commercial molecular platforms detect multiple pathogenic agents of gastrointestinal disease
  • Bacterial, viral, and parasitic targets
  • Rapid and sensitive results
  • Customizable PCR capability

• Despite technological advances, labs should maintain expertise and proficiency in conventional microscopy-based diagnostics
Questions??