

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

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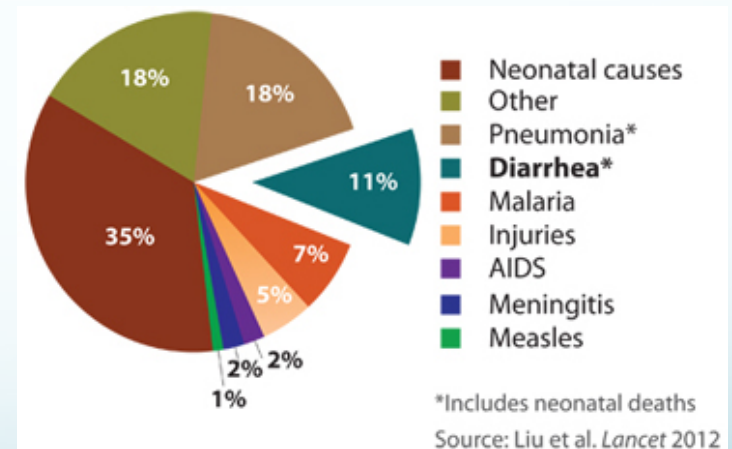
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Objectives

- 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

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+) add:

- **Test for:**
- *Microsporidia, Mycobacterium avium complex, Cytomegalovirus*

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

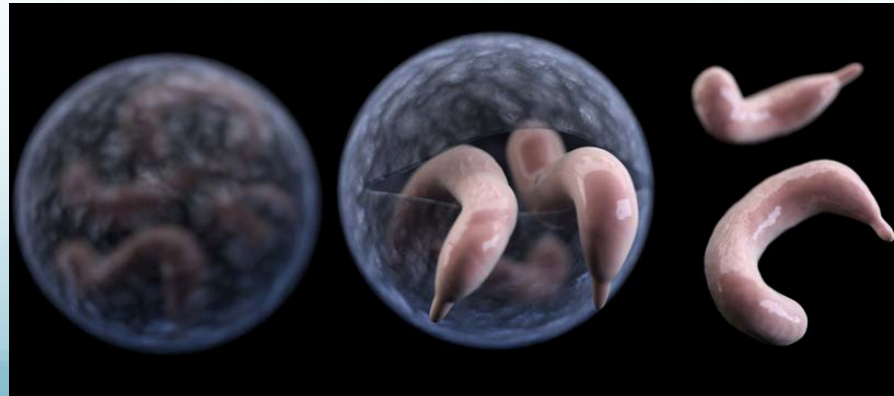
Giardiasis

- 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

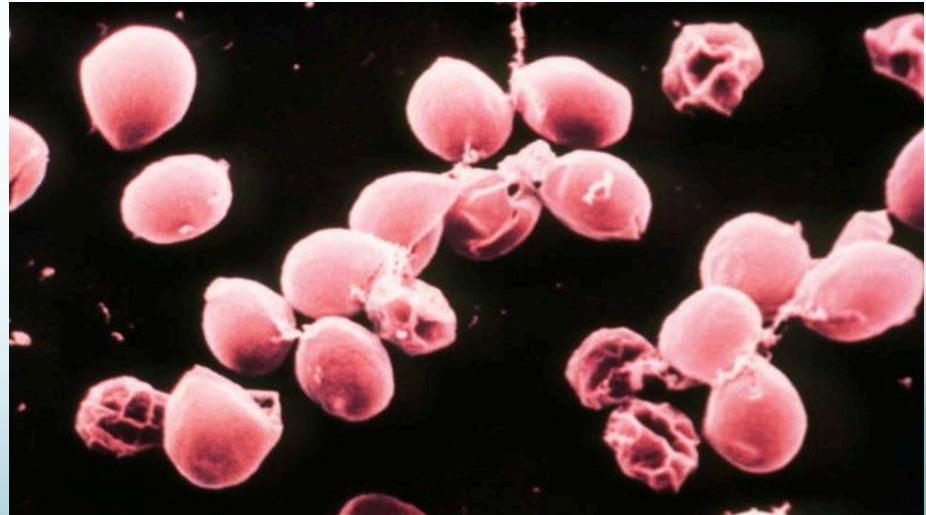


Cryptosporidium epidemiology

- 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

Cryptosporidiosis

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

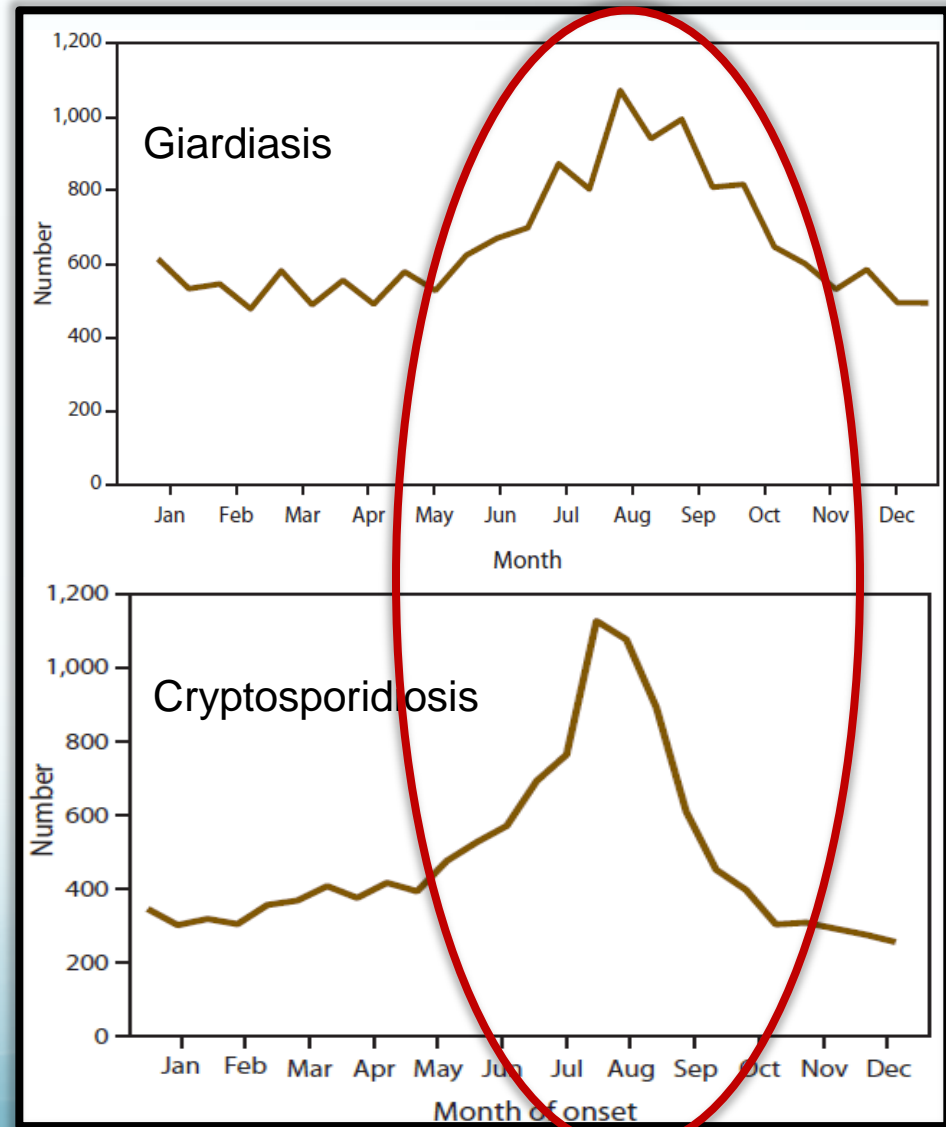


Crypto & HIV

- 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)

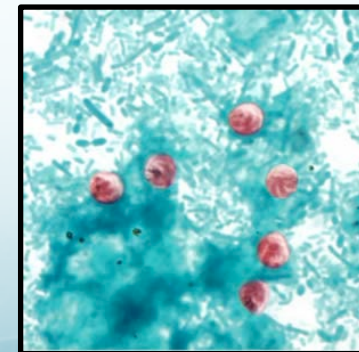
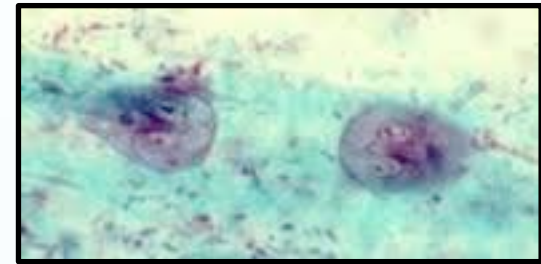
Disease seasonality

- 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



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

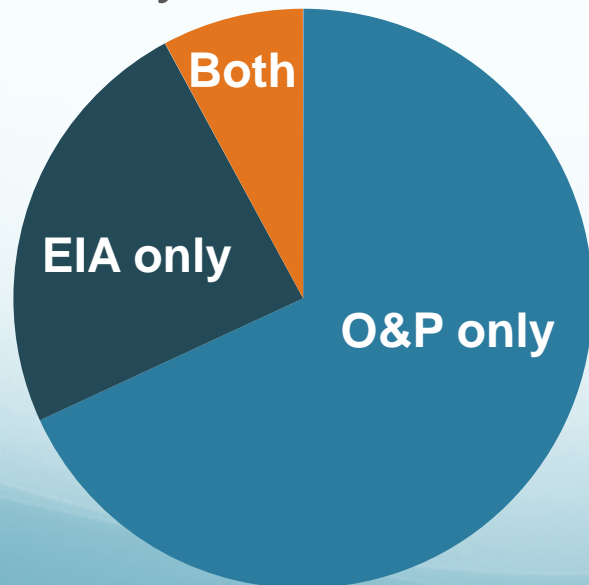
- O&P exams aren't all-inclusive and additional tests (e.g. MAF stain) may be needed for some pathogens
 - Conventional staining is only ~50% as sensitive as direct fluorescence assay (DFA) for these two pathogens*
- VERY time-consuming; labor intensiveness increases overall costs
- Highly skilled technical expertise required for reading slides
- Reliant on proper collection and preservation
- Usually unable to differentiate *E. histolytica* and *E. dispar*

Physician-based surveys: Use of parasite tests

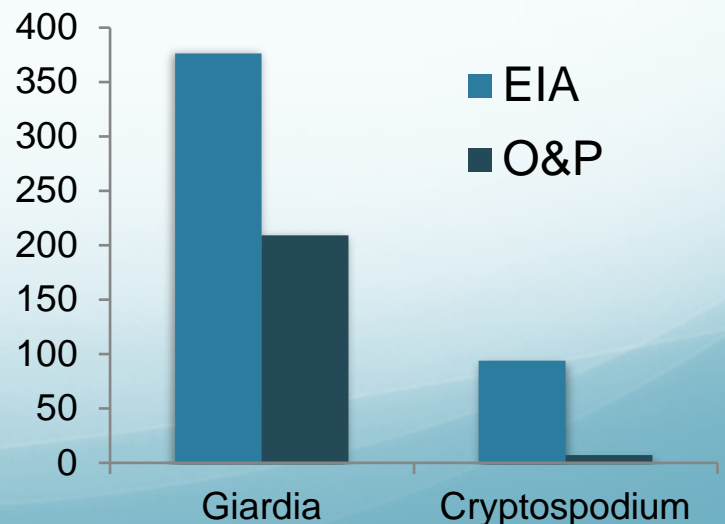
- <25% of physicians surveyed in Connecticut ordered appropriate test for *Cryptosporidium* despite suspicion of cryptosporidiosis
 - Morin *et al* 1997 Arch Intern Med 157:1017-1022
- 76% of respondents assumed *Cryptosporidium* testing was included with O&P exams
 - Hennessy *et al* 2004 CID 38(Suppl 3):S203-S211
- 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



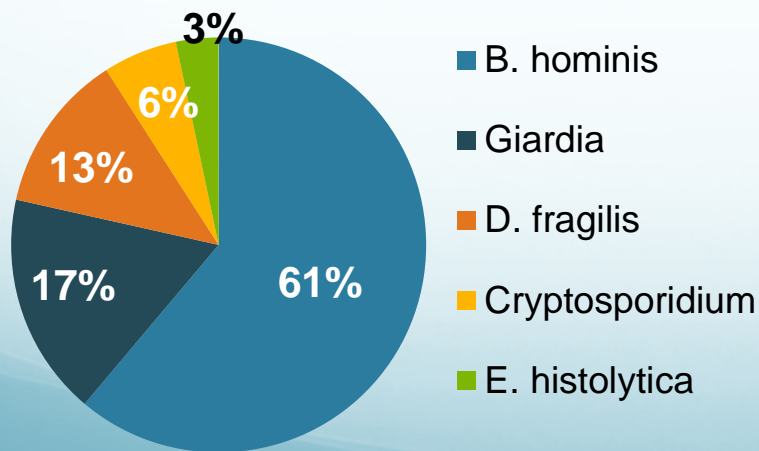
episodes positive by method



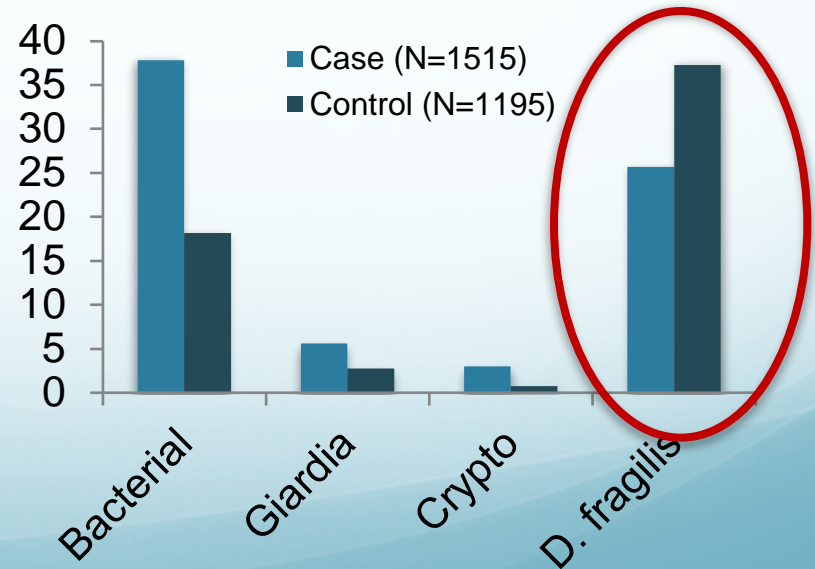
Things to consider for “non-conventional” enteric parasite diagnostics...

- 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



Case Control Study (Netherlands*)



Overview of diagnostic methods

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

Overview: Immunoassays

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

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:

Organism	Sensitivity	Specificity
<i>Giardia</i>	100%	100%
Crypto	97.3%	100%



MERIFLUOR *Cryptosporidium/Giardia*

- Specimen type:
 - 10% formalin, SAF
 - ExoFix
- Time to results: 10 min
- Performance:

Organism	Sensitivity	Specificity
<i>Giardia</i>	100%	100%
Crypto	97%	94%



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:

Sensitivity	Specificity
97.6- 100%	99.8-100%



Crypto Giardia Test Kit

- Specimen type:
 - Acceptable transport types not found on site
- Time to results: 30 min
- Performance:

Organism	Sensitivity	Specificity
<i>Giardia</i>	100%	N/A
Crypto	100%	N/A

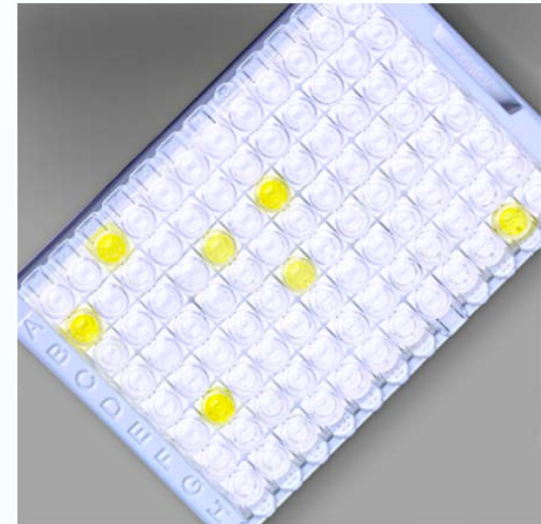
- Note: detects antigens specific for *G. lamblia* & *C. parvum*



ProSpecT Giardia/Cryptosporidium

- Specimen type:
 - Fresh/frozen unpreserved
 - 10% formalin, SAF, MF
 - Cary-Blair
 - Rectal swab/diapers
- Time to results: <2 hr
- Performance:

Sensitivity	Specificity
98.9%	99.6%



Xpect Giardia/Crypto

- Specimen type: stool
 - Fresh/frozen
 - 10% formalin
 - SAF
 - Cary-Blair or C&S
- Time to results: 15 min
- Performance:



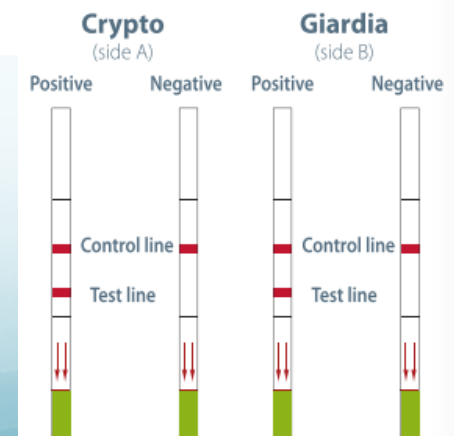
Organism	Sensitivity	Specificity
<i>Giardia</i>	95.8%	98.5%
Crypto	96.4%	98.5%

Crypto-Giardia Duo-Strip

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



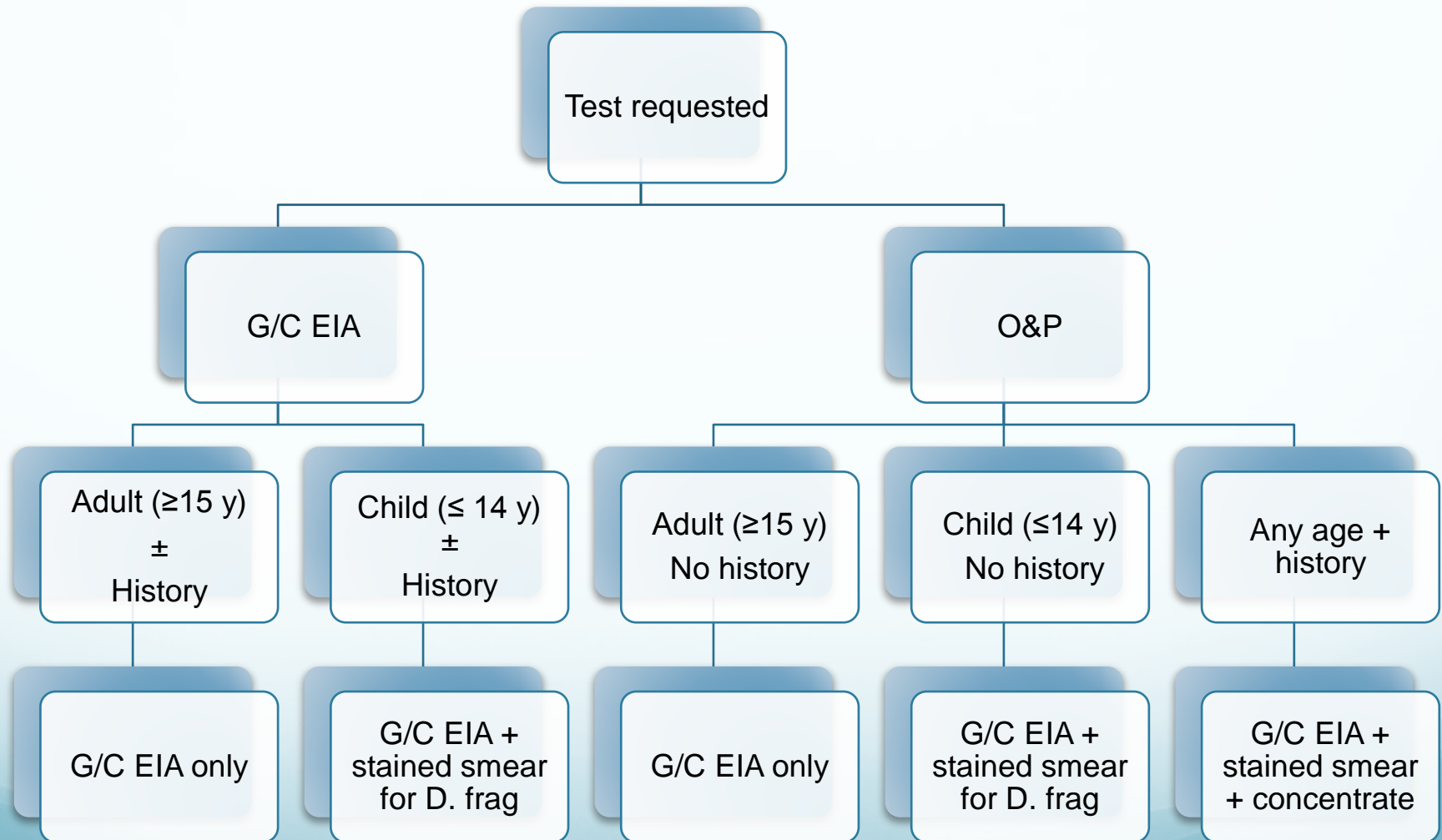
Organism	Sensitivity	Specificity
<i>Giardia</i>	100%	97.3%
Crypto	95.7%	100%



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



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)

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*

Method	% positive cases
Microscopy	24.2
Real-time PCR	38.3

Overview: commercial molecular assays

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

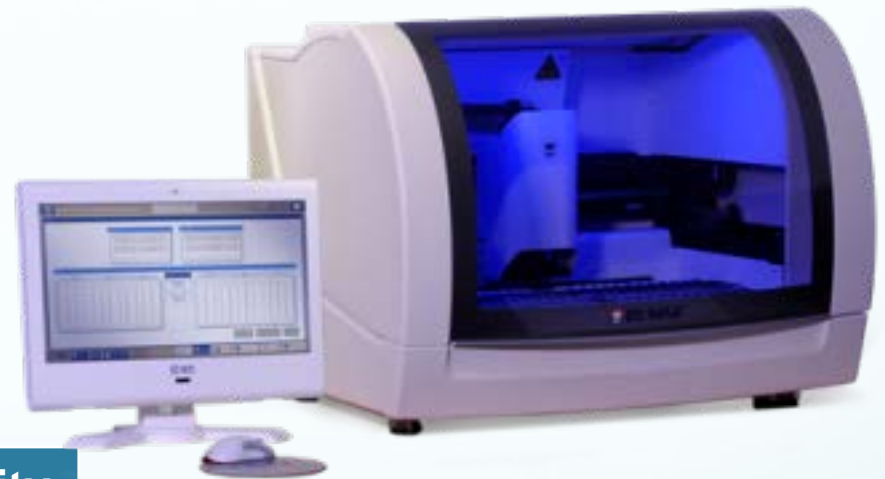
*Two panels: Enteric Bacterial Panel and Enteric Parasite Panel

Test menu on molecular platforms

BD MAX	FilmArray	Luminex xTAG
<ul style="list-style-type: none">•Enteric bacterial•Enteric parasite•GBS•<i>C. difficile</i>•MRSA•MRSA XT•StaphSR•Vaginitis•CT/GC/TV•CRE	<ul style="list-style-type: none">•GI pathogens•Resp. pathogens•Blood culture ID•Meningitis/ encephalitis	<ul style="list-style-type: none">•Enteric pathogens•Respiratory viruses•Multiple ASRs for individual targets (bacterial, parasitic, and viral pathogens)
LDT-capable	No LDT	LDT-capable

BD MAX Enteric Parasite Panel

- Specimen type:
 - Unpreserved
 - 10% formalin
- Time to results: ~3 hr
- Performance:



Organism	Sensitivity	Specificity
<i>Giardia</i>	95%	95%
Crypto	90.3-100%	99.8-100%

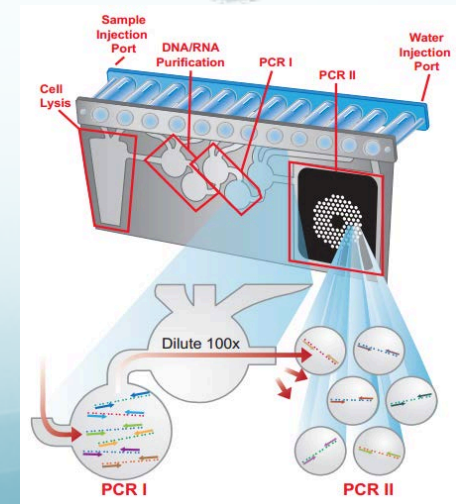
- Note: detects *G. lamblia*, *C. hominis* and *C. parvum*

FilmArray GI Panel

- Specimen type:
 - Cary-Blair
- Time to results: 1 hr
- Performance:



Target	Sensitivity	Specificity
Parasites	94.5-100%	99.5-100%



Luminex xTAG GPP

- Specimen type:
 - Fresh and frozen
 - Cary-Blair
- Time to results: <5 hr
- Performance:



Target	Sensitivity	Specificity
<i>Giardia</i>	100%	96.9-98.9%
Crypto	91.3-91.7%	100%

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??

