Issues Associated with Culture Independent Testing with Gastrointestinal Panels

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Objectives

- Discuss the advantages and disadvantages between culture based methods and molecular panels for gastrointestinal pathogen detection
- Compare results of traditional laboratory procedures to results from multiplex testing of gastrointestinal pathogens
- Explore Issues with culture independent testing.

Disclosure and Acknowledgements

- In 2013 UNMC received money from BioFire to perform testing for FDA approval for the GI panels. I personally was not paid nor my salary was paid but money was paid to our Pathology department.
- My salary is paid by the ELC supplemental Ebola grant from the CDC.

Gastrointestinal Disease

- Major cause of Morbidity and Mortality World-wide
- Diarrheal disease global concern with 2 billion cases a year
- 1.5 million deaths of children less than 5 of age.

Treatment for GI infections

- Oral/intravenous rehydration
- Antimicrobials maybe used in severe cases of gastroenteritis cased by certain pathogens.
- Timely and accurate diagnosis of stool pathogens is essential.

Gold Standard Method for Identification

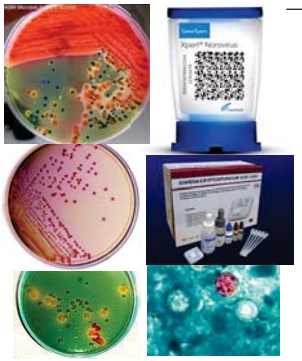
- Stool culture which require a 48 to 72 hours to complete.
- Enzyme-Linked Immunoabsorbant assay (EIA)based antigen detection.
- Microscopic detection
- Individual real-time PCR assay

Do we need a multiplex panel for gastrointestinal pathogens?

- Overlapping symptomology
  - **Norovirus:** diarrhea, nausea, vomiting, stomach pain, fever
  - ***Salmonella*:** diarrhea, stomach cramps, fever
  - ***Yersinia enterocolitica*:** diarrhea, fever, abdominal pain, vomiting
  - ***Cryptosporidium*:** diarrhea, stomach cramps, vomiting, fever
- Constant exposure to potential agents of gastroenteritis
- Each agent treated different—Some we can treat with antibiotics, relieve patient anxiety, rapid public health response



Do we need a multiplex panel for gastrointestinal pathogens?



TAT: 24 hours to 3 days
6 Test Orders

TAT: 2 to 24 hours
1 Test Order

Syndromic Testing

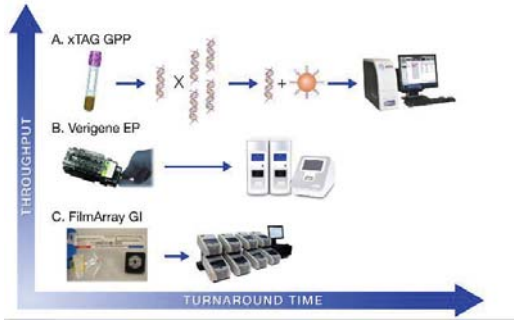
Allows the Health Care Professional to cast a wide net.

Multiplex (Syndromic) Panels

- Reduced Sample Size
- Broad coverage without ordering specific tests.
- Enhanced ability to pick up co-infections
- Higher Through Put
- And in some cases higher sensitivity

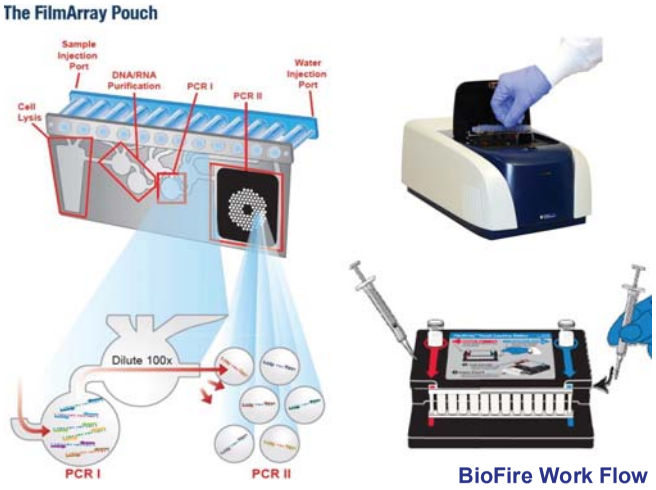
Multiplex Panels for Gastrointestinal Pathogens

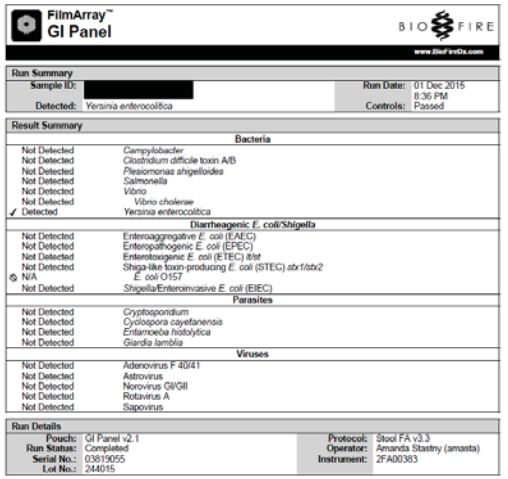
- FIVE FDA-cleared GI Panels
  - **Hologic** - *Salmonella*, *Shigella*, *Campy*, Shiga toxin positive *E. coli* (STEC)
  - **BD Max** - *Salmonella*, *Shigella*, *Campy*, STEC; Viral Kit; Parasite Kit
  - **Nanosphere** - *Salmonella*, *Shigella*, *Campy*, STEC, *Vibrio*, *Y. enterocolitica*, Norovirus, Rotavirus
  - **Luminex** - *Salmonella*, *Shigella*, *Campy*, STEC, O157, ETEC, *V. cholera*, *Y. enterocolitica*, *C. difficile*, Adenovirus, Norovirus, Rotavirus, *Cryptosporidium*, *E. histolytica*, *Giardia*
  - **BioFire** - Most comprehensive to date, 14 bacterial targets, 5 viral targets, 4 parasitic target



BioFire FilmArray™ Gastrointestinal Panel (GIP)

Bacteria	Diarrheagenic *E. coli*/Shigella	Parasites	Viruses
Campylobacter	Enteroaggregative *E. coli* (EAEC)	*Cryptosporidium*	Adenovirus F 40/41
Clostridium difficile toxin A/B	Enteropathogenic *E. coli* (EPEC)	*Cyclospora cayetanensis*	Astrovirus
Plesiomonas shigelloides	Enterotoxigenic *E. coli* (ETEC)	*Entamoeba histolytica*	Norovirus GI/GII
Salmonella	Shiga-like toxin-producing *E. coli* (STEC)	*Giardia lamblia*	Rotavirus A
Vibrio	*E. coli* O157		Sapovirus
Vibrio cholera	*Shigella*/Enteroinvasive *E. coli* (EIEC)		
Yersinia enterocolitica			





BioFire FilmArray™ GIP

- The GIP had a sensitivity of 100% for 12/22 targets
- All other targets had a sensitivity of >94.5%
  - Figures for *Vibrio*, *V. cholera*, and *E. histolytica* were not calculated due to low incidence rates
- Specificity for all analytes was >97%

Analyte	No. of detections[a] C	FA
Campylobacter spp.	35	58
C. difficile	165	204
P. shigelloides	3	18
Salmonella spp.	31	37
Vibrio spp.	0	2
V. cholerae	0	1
Y. enterocolitica	1	1
EAEC	83	109
EPEC	317	348
ETEC	22	31
STEC	33	38
E. coli O157	3	4
Shigella spp./EIEC (culture)[d]	49 (31)	49
Cryptosporidium spp.	18	24
C. cayetanensis	19	19
E. histolytica	0	0
G. lamblia	20	27
Adenovirus F 40/41	44	55
Astrovirus	7	8
Norovirus GI/GII	55	70
Rotavirus A	6	18
Sapovirus	46	59

C=comparator;FA=GIP

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BioFire FilmArray™ GIP

TABLE 6 Results of discrepant analysis

Analyte by FilmArray GI Panel result	Total no.	No. with investigation outcome[a]: Inconclusive	FA correct	FA incorrect
False positives				
Campylobacter spp.	24	5		19[d]
C. difficile toxin A/B	41			41
P. shigelloides	15			15
Salmonella spp.	6			6

- In total 199 of 237 GIP false positives were confirmed by an alternative method, confirming the positive result from the GIP
- Occasional cross reactivity between both ETEC and *Giardia* with commensal organisms
- Conventional culture routinely misses *P. shigelloides*, *Salmonella, Vibrio,* and *C. upsaliensis*
- Effective for the diagnosis of pathogens that were previously not routinely tested for such as diarrheagenic pathotypes of *E. coli*, Sapovirus, and Astorvirus

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Use of BioFire FilmArray™

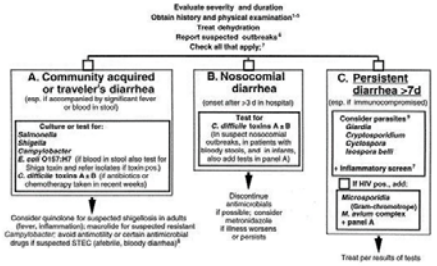
- **Advantages**
  - Increased sensitivity
  - Rapid turnaround time
  - Streamlines ordering procedures
- **Disadvantages**
  - Increased cost
  - Public health reporting necessitates organisms in culture

Implementation

- Stool culture and *Cryptosporidium/Giardia* EIA were discontinued and all other traditional testing was maintained
- Testing maintained in-house
  - Full ova and parasite (including modified acid fast)
  - Rotavirus
  - Norovirus
  - Adenovirus
- Also implemented an *Aeromonas* only culture
- Guidance document was written and circulated with results of all GIP testing

Practice Guidelines for the Management of Infectious Diarrhea
Richard L. Guerrant, Thomas Van Gilder, Ted S. Steiner, Nathan M. Thielman, Laurence Slutsker, Robert V. Tauxe, Thomas Hennessy,
Patricia M. Griffin, Herbert DuPont, R. Bradley Sack. Clin Infect Dis (2001) 32 (3): 331-351. DOI:https://doi.org/10.1086/318514

Figure 1



Implementation - Treatment Guidance

Table 1 – Etiology and Treatment Recommendations[4,9,10,11]

Pathogen	Common Presentation	Commonly Implicated Sources and Seasonality	Treatment Recommendations	Antibiotics (if indicated)
Bacteria				
Campylobacter	Fever, abdominal cramps, and diarrhea within 6-48 hours, fecal leukocytes often present	Poultry, unpasteurized milk and dairy products Peak season – spring, summer	Most patients recover without antimicrobial therapy. Antibiotics have been shown to reduce symptom duration by 1.3 days and are recommended for severe illness (high fever, bloody, severe, or worsening diarrhea) or risk factors for complications (elderly, pregnant women, immunocompromised).	Azithromycin 500 mg daily x 3 days Fluoroquinolone x 3 days* Immunocompromised patients may require prolonged therapy (7-14 days)
Clostridium difficile (toxin A/B)	More than 3 watery, loose, or unformed stools within 24 hours; lab findings may include leukocytosis and elevated creatinine	Recent antibiotic use, especially broad spectrum agents	Test not reported on panel. If CDI is suspected, order the *C. difficile* toxin assay.	Metronidazole 500 mg TID x 10-14 days Vancomycin 125 mg QID x 10-14 days See CDI treatment algorithm Discontinue antibiotics if possible www.nebraskamed.com/asp
Plesiomonas shigelloides	Severe abdominal cramps, and diarrhea within 6-48 hours	Fresh water, shellfish, international travel	Most patients recover without antimicrobial therapy. Unclear if antibiotics shorten the duration of illness. Consider in severe diarrhea, extremes of age, and immunocompromised.	Fluoroquinolone x 3 days* Azithromycin 500 mg daily x 3 days TMP/SMX DS BID x 3 days
Viruses				
Adenovirus F 40/41	Vomiting and non-bloody diarrhea within 10-51 hours	Children <2 yrs, day care	No therapy available. Treat symptomatically.	Antibiotics not indicated
Astrovirus		Children <1 yr, day care		
Norovirus GI/GII		Salads, shellfish, cruise ships, epidemic foodborne disease Peak season – winter		
Rotavirus A		Infants Peak season – winter		
Sapovirus		Children		

Patient Demographics

	Tests Run	Positive Tests*	% Positivity
Inpatient	703	161	23%
Outpatient	877	253	29%
Reference	636	195	31%
Public Health	39	26	67%

*Includes all analytes except *C. difficile*

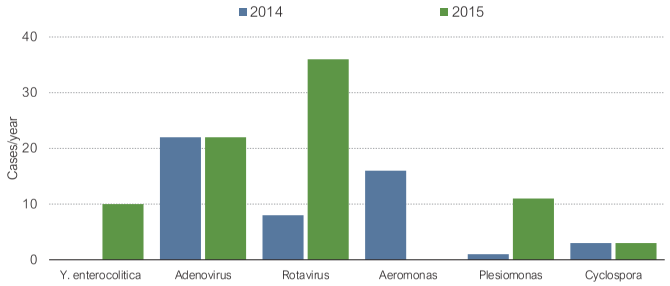
- Testing was distributed between inpatients, outpatients, reference clients (clinics, small hospitals throughout Western Iowa/Eastern Nebraska)
- Some testing was also run for public health purposes when concerns of an institutional outbreak were present

GIP and Traditional Methods
How Do They Compare

	2014	2015
Number of Tests	3417*	2255
Total Positive (%)	169 (4.9%)	370 (16.4%)
Salmonella	16	30
***Shigella*/EIEC**	3	15
STEC	8	32
Campylobacter	18	51
Norovirus	115	197
Giardia	7	17
Cryptosporidium	2	28

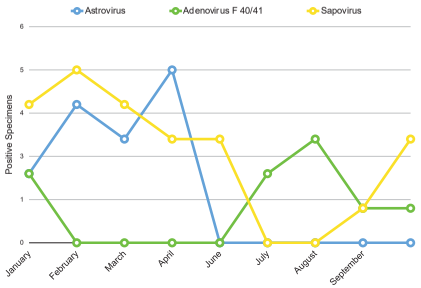
*Combined Stool culture, Giardia/Crypto EIA, Norovirus PCR

Increased Detection with New Methods



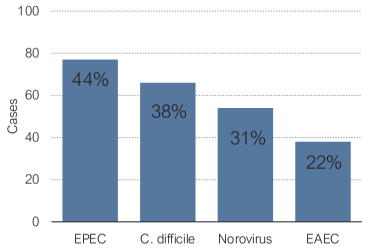
- Increased detection of all targets on the panel compared to traditional methods with the exception of *Cyclospora* and Adenovirus
- The absence of *Aeromonas* on the panel led to decreased detection
- Limitation: difficult to compare year to year due to seasonality, outbreaks

Prevalence of under diagnosed pathogens

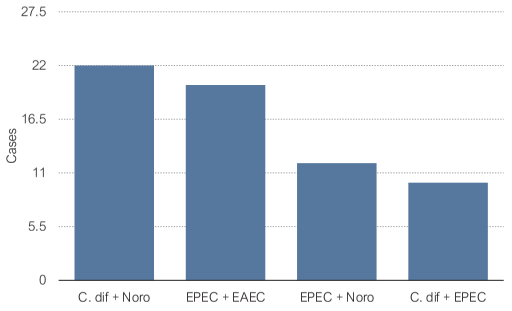


The high incidence of coinfection

- 19% (176/910) of positive panels had multiple targets detected
  - 2 targets detected in 15.9% of positive specimens
  - 3 or more targets detected in 3.3% of positive specimens



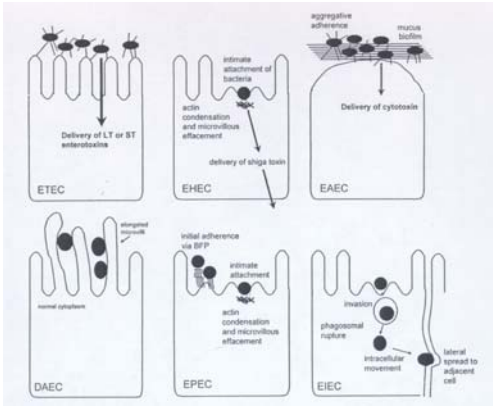
The high incidence of coinfection



- The most common coinfection observed was *C. difficile* and Norovirus
- The presence of diarrheagenic *E. coli* in many coinfections occurred primarily in the summer months

Diarrheagenic groups of *E. coli*

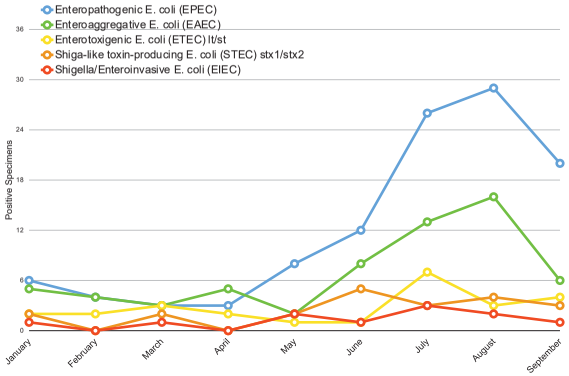
- Enterotoxigenic *E. coli* (ETEC)
- Enteroinvasive *E. coli* (EIEC)
- Enteropathogenic *E. coli* (EPEC)
- Enteroaggregative *E. coli* (EAEC)
- Diffusely adherent *E. coli* (DAEC)
- Enterohemorrhagic *E. coli* (EHEC)
  - *Escherichia coli* O157:H7



How do you interpret this result?

- EPEC
- ETEC
- EAEC
- *Salmonella*
- STEC

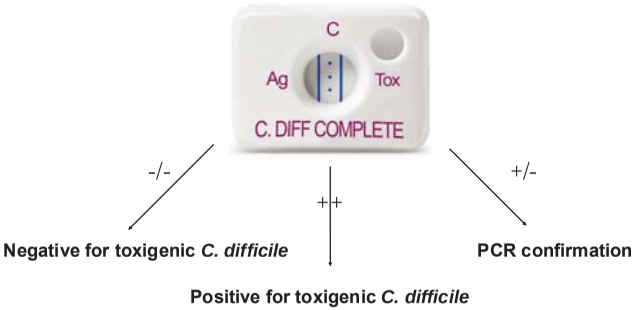
Seasonality of Diarrheagenic *E. coli*



The trouble with *Clostridium difficile*

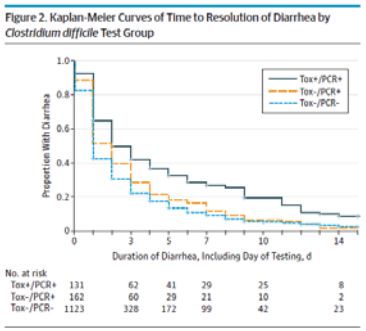
- *Clostridium difficile* was the only target on the panel not routinely reported
- *C. difficile* diagnosis is typically done by two different methodologies
  - PCR to detect microbial DNA
  - Enzyme immunoassay to detect presence of toxin and antigen
- Reviewed literature showing the association of morbidity and mortality with toxin production. Overall, *presence of toxin associated with increased morbidity and mortality, not presence of DNA*. Toxin negative, PCR positive patients difficult to assess.
- Opted to maintain our traditional *C. difficile* testing algorithm
  - When *C. difficile* was detected and no order was placed for *C. difficile* testing the provider was verbally notified of the result

C. difficile Testing



- Molecular testing cannot distinguish between asymptomatic colonization and symptomatic infection

Why not a solely molecular approach?



- Symptom duration and CDI complications for toxin - and PCR + patients resembles patients with no detectable *C. difficile*.

Comparison of *C. difficile* diagnosis using multiple platforms

GIP Result	Antigen	Toxin	*C. dif* Confirm	Total
+	+	+	N/A	88
+	+	-	+	102
+	+	-	-	16
+	-	-	N/A	47
+	no additional testing ordered			86

- Most common target amplified on the GI panel 15% (342/2255)
- Only 35% of GIP + samples that were tested by the traditional algorithm were toxin positive
- As has been documented, molecular testing has increased sensitivity compared to EIA
- Will continue to evaluate the most appropriate way to test and report *C. difficile*

Effect of CIDT on Public Health

- Rapid detection, increased sensitivity, and ability to detect more pathogens
- Public Health Laboratories require organisms in culture for confirmed diagnosis
  - Loss of subtyping and other follow up testing (e.g. PFGE)
  - Shift to whole genome sequencing will still require an isolate
  - Local outbreaks may be easier to detect; tracking necessary for widespread outbreaks
  - PulseNet data that is used to search for cluster identification and confirmation of outbreaks
- Loss of antimicrobial susceptibility testing

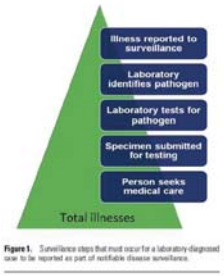
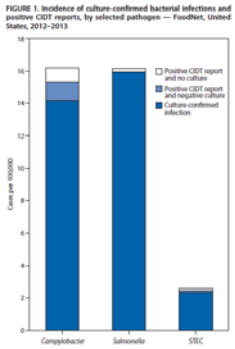


Figure 1. Surveillance steps that must occur for a laboratory-diagnosed case to be reported as part of notifiable disease surveillance.

JAMA Intern Med. 2015;175(11):1792-1801.

Public Health and the GI Panel

- Consultation with the Nebraska Public Health Laboratory resulted in an appropriate algorithm for follow up testing when necessary
- As other institutions in the state implement CIDT, Nebraska Medicine is performing recovery cultures of appropriate pathogens
  - *Salmonella*
  - STEC
  - *Yersinia enterocolitica*
  - *Vibrio*
- *Shigella* and *Campylobacter* are recovered on the patient account for appropriate susceptibility testing



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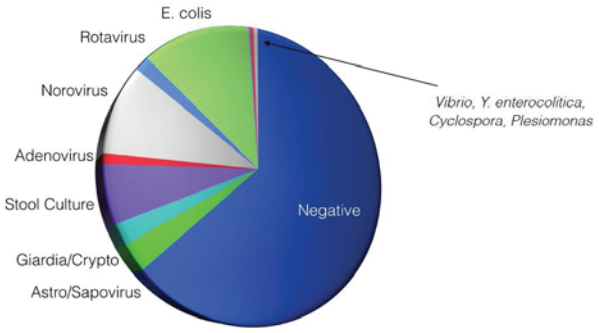
Isolate Recovery from the GIP

- State actively monitoring sites where CIDT may be adopted
- Recommend follow up culture for confirmation or forwarding specimen to Nebraska Medicine for culture
- Prioritize *Salmonella* and STEC culture

Organism	Detected	Recovered (%)
Campylobacter	51	35 (69)
Shigella/EIEC	15	10 (67)
Salmonella	30	23 (77)
STEC	22	9 (41)
STEC O157	5	3 (60)
Vibrio	3	2 (67)
Y. enterocolitica	10	3 (30)

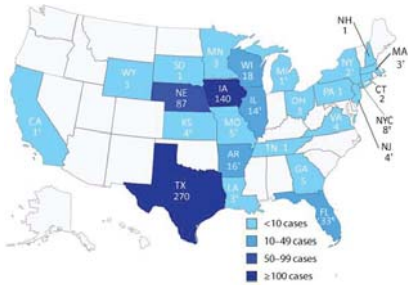
Do we need a multiplex panel for gastrointestinal pathogens?



Is there value in assays detecting rarely seen pathogens?

Case #1: An unexpected outbreak

- Day 1 of GI Panel testing for FDA studies
  - Cyclosporiasis Outbreak
  - Multiple outbreaks affecting 25 states with 631 confirmed cases

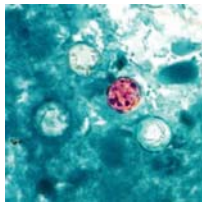


Case #2: A complex case of gastroenteritis

- 71 year old female transferred to Nebraska Medicine due to a pelvic fracture
- Also presented with acute kidney injury
- Reported a 2 week history of diarrhea
- GI Panel was ordered
  - *Vibrio cholera*, *Plesiomonas*, EPEC detected
- Blood cultures were drawn
  - Positive for *Edwardsiella* - also recovered from stool
  - Subsequent blood culture was positive for *Bacteroides*

Cyclospora cayetanensis

- Common in tropical and subtropical regions
- Spread through the ingestion of contaminated food or water.
  - First identified as a foodborne pathogen in the mid 1990s
- Not passed person to person
- In the absence of treatment, symptoms may last up to a month and relapses are common
- Traditional diagnosis through modified acid fast staining
- Ability to link *C. cayetanensis* cases is still developing



Patient History

- Patient had recently returned from a trip in South Dakota
  - Suspicion for *Vibrio* was low
- Following multiple inquiries and recovery of *V. cholera* in stool culture the patient's husband recalled eating raw oysters in a local establishment
- Epidemiological investigation determined that the oysters were Blue Point oysters that likely originated in the Chesapeake Bay area

http://www.cdc.gov/nationalsurveillance/cholera-vibrio-surveillance.html

Cyclospora cayetanensis

TABLE 1 Ordering practices related to the stool specimens positive for *Cyclospora* using the BioFire FilmArray GI panel[a]

Time of specimen collection in relation to outbreak[b]	No. of specimens for which *Cyclospora* testing was or was not ordered by provider	
	Ordered	Not ordered
Before (before 28 June)	0	3
Early (between 28 June and 12 July)	3	5
After outbreak established (after 12 July)	8	0

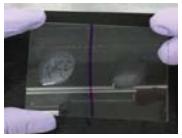
[a] For investigational use only.
[b] The Centers for Disease Control and Prevention became aware of two domestically acquired, laboratory-confirmed cases of *Cyclospora* infection on 28 June (1).

- Loss of proficiency at parasite exam at many clinical laboratories lead to a backlog of specimens and delayed diagnosis
- Between 2014 and 2015 the incidence of *Cyclospora* was the same between traditional methods and GIP testing
- Another test site showed a 40% increase in diagnosis between the culture and GIP for a *Shigella* outbreak

J Clin Microbiol. 2013 Nov;51(11):3909.

Vibrio cholera

- Toxin PCR was performed at UNMC and subsequently at the CDC
  - The isolate was found to be nontoxigenic
- Serotyping O1 and O139 was performed at NPHL and the CDC
  - O141 and O75 are found in the US and produce mild gastroenteritis
- The isolate has a unique PFGE pattern in their limited database (1200 isolates)
- In 2014, we had 5 orders for *Vibrio* with 0 recovered, in 2015 we detected 3 specimens positive for *Vibrio*



At the end of February 2017 this sleepy little town got sick.

Beaver City Gastrointestinal Illness Survey

The Nebraska Department of Health and Human Services (NDHHS) and South...

Case #3

21 people in Beaver City, NE with gastroenteritis was picked up by CITD

- The first few patients were seen at a local clinic and samples sent to the near by critical access hospital. They do have a full microbiology but stools are primarily done by BioFire. Then 10 other patients in a course of two days.
- All twenty one people had Campylobacter jejuna.
- Southwest Public Health and the Department of Health and Human Service of Nebraska set up a survey to get to the bottom of this.
- A contaminated well on a farm that leeched into the groundwater was suspected to be the cause.
- According to FoodNet, the percentage of *Campylobacter* diarrheal illnesses diagnosed only by CIDTs increased from 13% in 2012-2014 to 24% in 2015.
-

Evaluation of the BioFire FilmArray® Gastrointestinal Panel in a Midwestern Academic Hospital

- Studied published in December of 2016 in the European Journal of Clinical Microbiology Infectious Disease.
- Total of 2257 stools collected from January to December of 2015 were tested using the GIP.
- GIP detected one pathogen in 911(40%).
- Coinfections were detected in 176 (7.8%).
- The most frequently detected pathogens were C. difficile (15.2%), norovirus (8.9%), enteropathogenic *Escherichia coli* (7.1%), enteroaggregative *E. coli* (3.4%), *Campylocbacter spp*. (2.3%) and sapovirus (2.0%).

Recovery of bacteria from the Midwestern Academic Hospital for P/206ublic Health investigations varied

- 77% for *Salmonella*
- 30% for *Yersinia enterocolitica*.
- For stools positive for *C. difficile* on GIP that were tested by EIA, only 42.7% (88/206) were found to be producing the toxin.

Conclusions

- Implementation of culture independent testing leads to increased detection of many causes of gastroenteritis and improved turnaround time
  - Further studies needed to evaluate improvements in patient care, infection control practices, and appropriate antibiotic treatment
- Increased awareness of previously untested or under ordered pathogens
  - Abundant coinfections may indicate important areas for further investigation
- Potential to rapidly diagnose outbreak associated infections
- Comprehensive testing will hopefully decrease unnecessary testing and provide patient and provider with a diagnosis
- Changes in the workforce might necessitate testing that requires less hands on time and less technical specialty