Comparison of Multiplex Gastrointestinal Pathogen Panel and Conventional Stool Testing for Evaluation of Patients with HIV Infection

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Key points: Our retrospective study investigating the impact of multiplex gastrointestinal pathogen panels vs conventional stool testing in HIV patients identified significantly faster turnaround times, higher coinfection rates, and avoidance of unnecessary anti-infective therapy for viral infections.
ABSTRACT

**Background:** Gastrointestinal pathogen panels (GPP) are increasingly used to identify stool pathogens, but their impact in people living with HIV (PWH) is unknown. We performed a retrospective cohort study comparing GPP and conventional stool evaluation in PWH.

**Methods:** We included all PWH who underwent GPP (Biofire Diagnostics®; implemented September 15, 2015) or conventional testing, including stool culture, *Clostridium difficile* polymerase chain reaction (PCR) testing, fluorescent smears for *Cryptosporidium* or *Giardia*, and ova and parasite exams (O&P) from 2013-2017. A total of 1,941 specimens were tested with 169 positive specimens detected in 144 patients. We compared result turnaround time, pathogen coinfection, antibiotic treatment, and treatment outcomes between positive specimens detected by conventional testing versus GPP.

**Results:** Overall, 124 patient samples tested positive by GPP compared to 45 patient specimens by conventional testing. The GPP group demonstrated a higher co-infection rate (48.4% vs 13.3%; p<0.001) and quicker turnaround time (23.4 vs 71.4 hours; p<0.001). The GPP identified 29 potential viral infections which were undetectable by conventional stool tests. Unnecessary anti-infective therapy was avoided in 9 of 11 exclusively viral infections. Exclusively nonpathogenic parasites (N=13) were detected by conventional stool tests, the majority of which were treated with metronidazole. There were no significant differences in clinical outcomes between groups.

**Conclusions:** In PWH, GPP implementation improved antibiotic stewardship through shorter turnaround times and detection of enteric viral pathogens.
INTRODUCTION

Diarrhea is a prevalent gastrointestinal symptom in people with HIV (PWH) which may lead to increased morbidity and mortality [1,2]. Rapid identification of potentially treatable causes of diarrhea is particularly important in immunocompromised persons. Deficient cellular immunity puts PWH at increased risk for opportunistic parasitic infections such as Cryptosporidia and Microsporidia, while high-risk sexual behaviors have been linked to Shigella and other relapsing infections [3,4]. It is also important to identify potential causes of diarrhea in which antibiotic treatment is not indicated such as Shiga-like toxin producing E. coli (STEC) or Norovirus. In addition, persons with immunodeficiencies can have infectious gastroenteritis and not present with classical symptoms making definitive diagnosis a challenge. The differential diagnosis in immunosuppressed persons is often broad and can require multiple tests which take days to result, including bacterial cultures and examinations for ova and parasites. A lengthy infectious work up is required before non-infectious causes such as malabsorption (HIV enteropathy, lactose intolerance), medications or supplements are considered [5].

Prior to the use of multiplex nucleic acid tests, approximately 80% of acute gastroenteritis cases (suspected foodborne) had no detected pathogen [6]. When compared to conventional methods, multiplex nucleic acid testing demonstrates faster turnaround times and thus more rapid diagnosis, treatment and improved clinical sensitivity [7-16]. Although, the use of multiplex gastrointestinal pathogen panels (GPP) has increased over the past five years, the impact of these tests on PWH has not been investigated. To better understand the impact of GPP implementation, we compared the testing and treatment of diarrheal illnesses in PWH before and after the introduction of multiplex GI panel testing.
METHODS

Study Design:
We performed a retrospective cohort study involving PWH presenting with gastrointestinal symptoms to the University of California, San Diego (UCSD). Patients were included if they had a diagnosis of HIV (based on documented HIV viral load PCR and/or HIV 1/2 antibody ELISA), were seen at UCSD between September 15, 2013, and September 15, 2017, and underwent stool testing for suspected symptoms of infectious gastroenteritis (diarrhea, blood in stool, fever, nausea/vomiting). UCSD implemented multiplex GI panel testing September 15, 2015. This study was approved by the UCSD Institutional Review Board (IRB #181404).

Enteric Pathogen Testing: At our institution, infectious evaluation for diarrhea was performed exclusively using conventional stool testing (stool culture, *Clostridium difficile* PCR, *Cryptosporidium* smear (auramine stain), *Giardia/Cryptosporidium* Fecal Direct Fluorescent Antigen, ova and parasite exam (O&P) and trichrome stain) until September 2015. The *C. difficile* PCR test at our institution (Simplexa, Focus Diagnostics) detects *C. difficile* toxin B gene (tcdB). In September 2015, our institution implemented the GPP, BioFire FilmArray GI Panel® (BioFire Diagnostics, Salt Lake City, UT), for both inpatient and outpatient diarrhea evaluation. This test detects nucleic acid from 22 pathogens (13 bacteria, 5 viruses, and 4 parasites). All persons with a positive enteric pathogen test from September 2013-September 2017 were included for the current analysis. Persons with more than one positive result over the four year period were considered separate events if a new pathogen was detected.

Data Abstraction: We abstracted the following information from the medical records: (a) demographics: age, sex, ethnicity; (b) gastrointestinal disease characteristics: fever, diarrhea,
nausea/vomiting, hematochezia, other; (c) laboratory results: CD4 T-cell count, HIV viral load; (d) enteric pathogen testing: test type, pathogen(s) identified, turnaround time to result; (e) treatment characteristics: gastroenteritis treated with targeted anti-infective therapy (initiated in response to results), empiric anti-infective therapy (initiated before results available), anti-infective therapy exposure in the past 30 days, antiretroviral therapy (ART) status; and (f) outcomes: symptom resolution at 7 days and 30 days, diagnostic interventions (imaging, endoscopy, biopsy/cytology), hospitalization, and surgery.

**Statistical analysis:** We compared GPP and conventional testing using Fisher’s exact test for categorical variables and Wilcoxon Rank Sum test for continuous variables. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed with statistical software R (version 3.5.1).

**RESULTS**

**Patient Characteristics**

A total of 1,941 specimens were tested in PWH (n=1,705 conventional stool tests; n=236 GPP) with 169 positive specimens detected in 144 patients from Sept 2013-Sept 2017 (n=45 conventional stool testing; n=124 GPP). Seventeen patients had two separate positive pathogen tests and four patients had three positive pathogen tests over the four year time period. Of this group, 10 patients had repeat infection with a previously identified pathogen. The average time elapsed between test dates was 344 days (range 42-856 days) with four repeat infections in less than 3 months. Baseline patient demographics and clinical presentation characteristics are summarized (Table 1). PWH with positive pathogen testing at our institution consisted predominantly of males (97%). The majority of persons presented
with diarrhea (90.5%); 4% presented with hematochezia, 7.1% presented with fever and 10.7% presented with nausea and/or vomiting. The overall mean CD4 T-cell count was 520 cells/mL (SD=332). Persons with a positive conventional stool test had significantly lower CD4 T-cell counts at presentation compared to persons with a positive GPP (372 vs 574 cells/mL, p<0.001), however, CD4 T-cell counts were overall much higher after 2015. Most persons were taking ART (82.2% in conventional stool test group vs 85.4% in GPP group, p=0.634) and there was no difference in HIV viral load (p=0.49).

**Enteric Pathogen Testing**

Overall, 124 PWH tested positive using GPP. The most common enteric pathogens detected by GPP were *E. coli* species (Table 2). Coinfection was higher in the GPP group with up to four pathogens detected in a single patient sample compared to standard stool testing (48.4% vs 13.3%, p<0.001). The GPP positivity rate among PWH was 52.5% (124 positive GPP/236 total GPP), higher than the overall observed rates at our institution, 37.1±2.2% (BiofireTrend Reports). Among the 45 positive samples by conventional stool testing, *Clostridium difficile* was the most common enteric pathogen detected (26.6% of positive specimens; 2.2% of total specimens). Before 2015, conventional ova and parasite testing often reported nonpathogenic protozoa, including *Entamoeba coli* (N=7), *Entamoeba hartmanni* (N=2), *Endolimax nana* (N=7), *Iodamoeba butchilii* (N=2) and *Blastocytis hominis* (N=6).

The multiplex panel allowed for the identification of potential pathogens which could not be diagnosed with conventional testing. These included Enteroaggregative *E. coli* (N=34), Enterotoxigenic *E. coli* (N=5) and Enteropathogenic *E. coli* (N=40). Twenty-nine potential viral etiologies were detected in 28 different persons (Adenovirus, N=2; Astrovirus, N=2; Norovirus, N=22; Rotavirus, N=1; Sapovirus, N=2), which would not have been identified
before 2015. Not surprisingly, the implementation of the GPP at our institution decreased the utilization of conventional stool cultures by 66% (158 to 56 total cultures upon GPP implementation). Utilization of Cryptosporidium smear and ova and parasite exam were also dramatically decreased upon GPP implementation (262 vs 96 Cryptosporidium smears and 273 vs 91 O&P exams).

The overall mean turnaround time for positive results was significantly decreased with the institution of multiplex testing compared to conventional testing (23.4 vs 71.4 hours; p<0.001) (Table 3). Detection of Giardia was decreased to an average of 22.4 hours with GPP as opposed to 74.8 hours with conventional stool studies (p<0.001). Campylobacter and Salmonella, two highly infectious bacterial species, were detected within 22.4 hours versus 56.5 hours for conventional testing (p=0.038) and 10.7 hours versus 68 hours, respectively. The mean turnaround time for Shigella/EIEC was 19.3 (SD=11.4) hours. Twelve of the 22 Shigella/EIEC were confirmed with culture. No Shigella sp were detected by conventional stool tests.

**Anti-infective Therapy**

A summary of anti-infective therapy characteristics is included in Table 4. Of the positive specimens, a total of 134 of 169 (79.3%) patients received anti-infective therapy (75.6% of conventional stool tests and 80.7% GPP, p=0.5). Of patients who received antibiotics, 21.3% were empirically treated while 58.0% received targeted treatment once test results were known. Of the patients empirically treated, 12 (34.3%) were continued on the same treatment once results were known. There were no differences between groups for antibiotic exposure.
or patients on prophylactic anti-infective therapy. CD4 T-cell count, HIV viral load and ART status did not impact receipt of empiric antibiotic treatment. Empiric treatment between the positive GPP and negative GPP groups demonstrated no significant difference (21.3% of positive GPP receiving empiric treatment vs. 18.1% of negative GPP receiving empiric treatment; p=0.549).

To identify the impact of the GPP on antibiotic stewardship, we evaluated the treatment of viral infections, *Enteroaggregative* and *Enteropathogenic E. coli*, and nonpathogenic parasites (i.e. *Entamoeba coli*, *Entamoeba hartmanni*, *Endolimax nana*, *Iodamoeba butchili*, and *Blastocystis hominis*). Exclusive viral infections (not including viruses co-identified with bacteria or pathogenic parasites) were detected in 11 persons, only two of whom (18.2%) received antibiotics. EPEC was detected in 40 of 124 (32.3%) specimens and 60% of those received treatment with Ciprofloxacin (mono-infection 50%, co-infection 65.4%; see Table 4). EAEC was detected in 34 of 124 (27.4%) specimens, 76.5% of whom received treatment with Ciprofloxacin, TMP-SMX or azithromycin (mono-infection 71.4%, co-infection 77.8%; see Table 4). Nonpathogenic protozoa were exclusively detected in 13 persons with 10 (76.9%) receiving anti-infective therapy (metronidazole n=7; mebendazole/albendazole n=1; ciprofloxacin n=1; ceftriaxone n=1). Three of 7 (42.9%) of *Entamoeba coli*, 0 of 1 (0%) *E. hartmanni*, 2 of 4 (50%) *E. nana*, 2 of 2 (100%) of *I. butchili*, and 4 of 5 (80%) of *Blastocystis hominis* persons were treated.

**Impact of Enteric Pathogen Testing on Clinical Outcomes**

We compared the clinical outcomes (symptom resolution at 7 and 30 days, respectively) and interventions (imaging, endoscopy, pathologic tissue evaluation and surgery) between
subjects with a positive conventional stool test and subjects with a positive gastrointestinal pathogen panel. There were no significant differences in clinical outcomes or interventions between groups (Table 5).

DISCUSSION

In this retrospective cohort study of 169 PWH who tested positive by either conventional stool testing or multiplex GPP for symptoms suggestive of infectious gastroenteritis, we made several fundamental observations. Of interest, coinfection rates of 48.4% among the GPP positive samples were higher than what has been reported in the literature for HIV seronegative persons (12%-33%), as well as the rate of co-infection overall observed at our institution (24.3%; BioFire Trend Reports) [7, 8, 11, 12, 13, 14]. The significance of coinfection is uncertain and warrants further investigation, particularly in PWH. The immunosuppression associated with HIV may result in prolonged shedding that potentially contributes to the increased detection of multiple pathogens. Prior studies have shown multiple pathogens are more likely to be detected in children less than five years of age [12-14]. Different coinfections have also been described in other studies [7,13]. One multinational study reported Campylobacter and EPEC as the most common coinfections while EAEC, Y. enterocolitica, and norovirus were detected most frequently by FilmArray in a separate study [7, 13]. The role and impact of these specific pathogen combinations is not well established or understood. Interestingly, EPEC, an organism classically associated with developing countries and diarrhea in children was the most frequently detected pathogen in our GPP group and in 43.3% (26 of 60) of coinfections.
Consistent with previous studies comparing multiplex PCR pathogen panels to conventional stool studies, turnaround time was significantly decreased by PCR methods [7-16]. Faster turnaround times in the GPP group (23.4 vs 71.4 hours; p<0.001) allowed decisions based on results within 24 hours in most patients. Rapid turnaround time may also allow faster implementation of infection prevention and isolation to decrease the risk of person-to-person transmission amongst hospitalized patients. Prior to the widespread use of nucleic acid testing, the detection of enteric viruses in PWH with diarrhea ranged from 7.4-45% [17-19]. This study demonstrates clinicians are appropriately deferring antibiotics in PWH with viral causes of infectious gastroenteritis (only 18.2% treated). Two areas we identified to improve antibiotic stewardship were the treatment of noninvasive E. coli in adults and nonpathogenic parasites. Although Enteroaggregative (EAEC) and Enteropathogenic E. coli (EPEC) are major causes of diarrhea in children, their importance in adults, even PWH is less clear [20-22]. Yet, 60% of persons with EPEC and 76.5% with EAEC received treatment with Ciprofloxacin or Azithromycin. Most persons with exclusive nonpathogenic protozoa (76.9%) received metronidazole, despite a reporting disclaimer as “nonpathogenic protozoan.” Increased training in appropriate antibiotic use is warranted.

Another interesting finding was the importance of rapid diagnosis of Shigella infection by GPP during an outbreak which occurred during the study period. A large multistate outbreak of Shigella infection in men who have sex with men was reported in 2015-2016 [13]. We detected 12 cases of culture confirmed Shigella sp from 2015-2017 with none in the previous two years in this population. Reflex culture confirmation of Shigella sp. in Shigella/EIEC PCR positive stools is important both for recovery of isolates to send to Public Health Laboratories [24] and for sensitivity testing, as increasing resistance to ciprofloxacin, trimethoprim-sulfamethoxazole, and azithromycin has been detected in Shigella sp [25].
Another example of rapid outbreak detection occurred in Iowa and Nebraska during evaluation of the FilmArray GPP. An outbreak of *Cyclospora* was detected by the FilmArray GPP one week before detection by conventional testing [14]. Biofire Trend was implemented to provide BioFire users with an up-to-date view of GI pathogens circulating at their institution compared to nationally [27]. This is achieved by providing de-identified test results to a cloud database that is available in real time on the Syndromic Trends public website [27]. Utilization of Biofire Trend could potentially help detect the beginning of GI pathogen outbreaks specifically in PWH presenting with symptoms of infectious gastroenteritis.

The cost effectiveness of multiplex PCR GPP has not definitively been established [28]. A study performed in the United Kingdom showed GPP assay use resulted in $34,800 in laboratory expense while reducing overall health care costs by $69,500 (when accounting for hospital days, isolation costs, etc.) [29]. Beal, et al. demonstrated a similar trend with increased laboratory expenses with net savings of $293.61 per patient when hospital stay and radiology costs are taken into account [30]. Unfortunately pursuing this question was beyond the scope of this study but should continue to be evaluated specifically considering the potential costs savings of appropriate anti-infective therapy.

We were unable to find any differences in clinical outcomes and interventions between conventional stool testing and GPP. This can be attributed to the subjectivity of symptom resolution in addition to persons being lost to follow up.

In conclusion, based on this retrospective cohort study, the utilization of GPP in PWH is highly advantageous for the rapid turnaround time, identification of viral infections which do
not warrant antibiotic treatment, and the early identification of potential outbreaks. Future studies should be performed to evaluate the significance of multiple pathogens detected by GPP in HIV patients. In addition, the use of GPP to detect outbreaks in PWH warrants further investigation. The cost effectiveness of multiplex PCR also warrants further investigation, particularly in the outpatient setting when hospital stay and isolation cost savings are noncontributory.

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| Characteristic                        | Conventional Stool Tests (n = 45) | GPP (n = 124) | p     |
|--------------------------------------|-----------------------------------|---------------|-------|
| Male, n (%)                          | 45 (100)                          | 119 (96.0)    | 0.326 |
| Mean age (+/- SD)                    | 47.2 (9.2)                        | 48.4 (10.2)   | 0.523 |
| Ethnicity, n (%)                     |                                   |               | 0.171 |
| White                                | 22 (48.9)                         | 72 (58.1)     |       |
| Black                                | 4 (8.9)                           | 10 (8.1)      |       |
| Hispanic                             | 18 (40)                           | 31 (25)       |       |
| Other                                | 1 (2.2)                           | 11 (8.9)      |       |
| Symptoms at presentation, n (%)      |                                   |               |       |
| Diarrhea                             | 41 (91.1)                         | 112 (90.3)    | >0.999|
| Blood in stool                       | 1 (2.2)                           | 6 (4.8)       | 0.676 |
| Fever                                | 1 (2.2)                           | 11 (8.9)      | 0.185 |
| Nausea &/or vomiting                 | 4 (8.9)                           | 14 (11.3)     | 0.783 |
| Labs at presentation                 |                                   |               |       |
| CD4 count, mean (+/- SD)             | 371.8 (264.8)                     | 574.3 (337.7) | <0.001|
| Viral load copies/mL, n (%)          |                                   |               | 0.486 |
| <50                                  | 31 (70.5)                         | 93 (75)       |       |
| 50-200                               | 2 (4.6)                           | 10 (8.1)      |       |
| ≥200                                 | 11 (25)                           | 21 (16.9)     |       |
| Receiving ART, n (%)                 | 37 (82.2)                         | 105 (85.4)    | 0.634 |

**Table 1:** Characteristics of patients with HIV infection with positive enteric pathogen testing using either conventional stool evaluation or multiplex gastrointestinal pathogen panel.
| Bacteria, *n* | Conventional stool evaluation (*n* = 45) | Gastrointestinal Pathogen Panel (*n* = 124) |
|--------------|----------------------------------------|------------------------------------------|
| CDI          | 11                                     | 29                                       |
| *Escherichia coli* species |                                      |                                          |
| EAEC         | -                                      | 34                                       |
| EPEC         | -                                      | 40                                       |
| ETEC         | -                                      | 5                                        |
| *Campylobacter sp*       | 2                                      | 13                                       |
| *Salmonella sp*          | 1                                      | 1                                        |
| *Yersinia sp*            | 0                                      | 1                                        |
| *Shigella/Enteroinvasive E. coli* | 0*                                     | 22*                                      |
| *Shigella sp* (confirmed) | 0*                                     | 12*                                      |
| *Aeromonas sp*           | 1                                      | 0                                        |
| *Mycobacterium sp*       | 1                                      | 0                                        |
| Viral, *n*              | 0                                      | 29                                       |
| Adenovirus             | -                                      | 2                                        |
| Astrovirus             | -                                      | 2                                        |
| Norovirus              | -                                      | 22                                       |
| Rotavirus              | -                                      | 1                                        |
| Sapovirus              | -                                      | 2                                        |
| Parasites, *n*          |                                        |                                          |
| *Cryptosporidium*       | 5                                      | 3                                        |
| *Giardia lamblia*       | 9                                      | 13                                       |
| Non-pathogenic parasites, *n* |                                      |                                          |
| *Entamoeba coli*        | 7                                      | 0                                        |
| *Entamoeba hartmannii*  | 2                                      | 0                                        |
| *Endolimax nana*        | 7                                      | 0                                        |
| *Iodamoeba butchilii*   | 2                                      | 0                                        |
| *Blastocystis hominis*  | 6                                      | 0                                        |
| Co-infections, *n (%)*  | 6 (13.3)*                              | 60 (48.4)*                               |

Table 2. Identification of enteric pathogens in patients with HIV detected by gastrointestinal pathogen panel or conventional stool testing.

*Indicates a significant difference in detection between GPP and conventional stool testing; *Shigella/Enteroinvasive E. coli* *p*=0.001; *Shigella sp* (confirmed) *p*=0.037; Co-infections *p*<0.001
| Turnaround time (hours) | Conventional Stool Tests (mean hours +/- SD) | N, conventional stool tests | GPP (mean hours +/- SD) | N, GPP | P  |
|-------------------------|-----------------------------------------------|-----------------------------|-------------------------|--------|----|
| Overall                 | 71.4 ± 59.7                                   | 44                          | 23.4 ± 16.9             | 124    | <0.001|
| Campylobacter sp        | 56.5 ± 17.7                                   | 2                           | 22.4 ± 9.2              | 13     | 0.038|
| Salmonella sp           | 68.0                                          | 1                           | 10.7                    | 1      | -   |
| Shigella/EIEC           | -                                             | 0                           | 19.3 ± 11.4             | 22     | -   |
| Cryptosporidium         | 77.0 ± 60.7                                   | 5                           | 27.9 ± 17.0             | 3      | 0.393|
| Giardia lamblia         | 74.8 ± 47.5                                   | 9                           | 22.4 ± 6.1              | 13     | <0.001|

Table 3. Turnaround time from collection to reporting of the key bacterial (Campylobacter sp, Salmonella sp, Shigella/EIEC) and parasitic (Cryptosporidium, Giardia lamblia) pathogens.
| Characteristic                                      | Conventional Stool Tests (%) | GPP (%) | P   |
|----------------------------------------------------|------------------------------|---------|-----|
| Empiric therapy                                    | 11 (24.4)                    | 25 (20.2) | 0.532 |
| Retrospective targeted therapy                     | 3 (27.3)                     | 9 (37.5) | 0.709 |
| Switched to targeted therapy                       | 4 (36.4)                     | 7 (28)   | 0.703 |
| Targeted therapy                                   | 23 (51.1)                    | 75 (60.5) | 0.294 |
| Pathogens of interest treated with anti-infective therapy |                          |         |     |
| EAEC mono-infection with targeted therapy          |                              | 5/7 (71.4) |     |
| EAEC co-infection with targeted therapy            |                              | 21/27 (77.8) |     |
| EPEC mono-infection with targeted therapy          |                              | 7/14 (50)   |     |
| EPEC co-infection with targeted therapy            |                              | 17/26 (65.4) |     |
| Viral infection                                    |                              | 2/11 (18.2)  |     |
| History of anti-infective therapy in past 30 days  | 15 (33.3)                    | 30 (24.2) | 0.243 |
| Receiving anti-infective prophylaxis               | 7 (15.6)                     | 10 (8.1)  | 0.159 |

**Table 4.** Treatment characteristics of HIV patients with positive enteric pathogen testing using either conventional stool evaluation or multiplex gastrointestinal pathogen panels.
| Clinical Outcomes and Interventions | Conventional Stool Tests (%) n = 45 | GPP (%) n = 124 | P |
|------------------------------------|--------------------------------------|-----------------|---|
| Symptom resolution at 7 days       | 2/32 (6.3)                          | 13/63 (20.6)    | 0.081 |
| Symptom resolution at 30 days      | 10/42 (23.8)                        | 34/111 (30.6)   | 0.433 |
| Interventions                      | 2 (4.4)                             | 8 (6.5)         | >0.999 |
| Imaging                            | 0 (0)                               | 1 (0.8)         | >0.999 |
| Endoscopy                          | 2 (4.4)                             | 6 (4.8)         | >0.999 |
| Biopsy &/or cytology               | 1 (2.2)                             | 3 (2.4)         | >0.999 |
| Surgery                            | 0 (0)                               | 1 (0.8)         | >0.999 |

Table 5. Comparisons of clinical outcomes and interventions between subjects with a positive conventional stool test and subjects with a positive gastrointestinal pathogen panel.