# Detection of *Clostridium difficile* Infection and Other Pathogens in Children with Diarrhea by FilmArray® GI Pouch

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## Figure 1. The FilmArray Instrument and Pouch

The FilmArray® is a lab-in-a-pouch medium-scale fluid manipulation system performed in a self-contained, disposable, thin-film plastic pouch. The FilmArray platform processes a single sample, from nucleic acid purification to result, in a fully automated fashion.

The FilmArray test is initiated by injecting re-hydration solution and a patient sample into the FilmArray pouch and placing it in the FilmArray instrument. The user enters the sample and pouch type (using a barcode reader) into the software and initiates a run. Results are provided in ~ 1 hour.

The FilmArray pouch has a fitment (see label A) containing all needed freeze-dried reagents and plungers (B) that plunge liquids to the film portion of the pouch. This portion consists of stations for cell lysis (blister C), magnetic-bead based nucleic acid purification (D & E), first-stage multiplex PCR (F & G) and an array of 102, second-stage nested PCRs (I).

PCR primers are dried into the wells of the array and each primer set amplifies a unique product of the first-stage multiplex PCR. The second-stage PCR product is detected in real-time using a fluorescent-double-stranded DNA binding dye, LCGreen®.

## INTRODUCTION

*Clostridium difficile* infection (CDI) is a leading cause of diarrhea and is linked to ~14,500 American deaths each year (CDC, 2012, Hall, 2012). It is estimated that 2-3% of healthy adults are carriers and that ~20% of hospitalized individuals acquire the bacteria during hospitalization (Medscape, 2012). The incidence and mortality due to CDI have been on the rise since 1990 (DuPont, 2010).

A FilmArray GI panel (FilmArray GI) is currently being developed for simultaneous detection of 26 bacterial, viral, and protozoal GI agents, including *Clostridium difficile* (*C. difficile*) (Figure 2). Identification of CDI is based on detection of tcdA and/or tcdB genes which produce an enterotoxin (toxA) and a cytotoxin (toxB), respectively. The hypervirulent NAP-1 (ribotype 027) strain is identified by detecting a deletion in the tcdC gene which codes for the transcriptional repressor of tcdA and tcdB.

The overlapping clinical presentation of a broad range of diarrhea-causing pathogens makes multi-target testing extremely valuable in aiding to diagnose the causative agent(s). This approach is contrary to the current paradigm for CDI testing which most often involves testing the patient for *C. difficile* only. This decision is based on the clinical representation in association with the likelihood of a hospital-acquired infection and/or antibiotic treatment. In patients suspected with CDI physicians may question the value of a multi-target testing for additional GI pathogens.

The rationale for the inclusion of a *C. difficile* test in the multi-target FilmArray GI panel was evaluated in this study by comparison of the positivity yield for *C. difficile* and other pathogens in three categories of specimens: (1) those where CDI was strongly suspected and only testing for *C. difficile* was requested, (2) those where CDI was suspected together with other infections and the testing was requested not only for *C. difficile* but also for at least one other pathogen, and (3) those where CDI was not suspected and testing for *C. difficile* was not requested (see Figure 3).

## METHOD

- Samples were stool from children with diarrhea submitted for any test to Primary Children's Medical Center (PCMC), Salt Lake City
- Specimens submitted August 2010- March 2012
- Tests ordered at physician discretion (1 and 7 tests per specimen)
- Illumigene *C. difficile* (Meridian Diagnostics) used in PCMC clinical lab
- Subset of 264 specimens were assessed for the presence of 26 pathogens with the FilmArray GI panel:
- Divided into 3 categories based on tests ordered

**Figure 3. PCMC Specimen Collection by Testing Category**

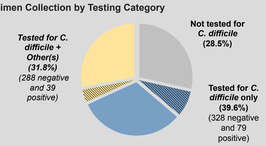



**Table 1. PCMC Specimens Tested in FilmArray GI**

| Testing Category | *C. difficile* by Illumigene | # Retested in FilmArray GI |
|---|---|---|
| Not tested for *C. difficile* Category 3 | N/A | 72 |
| Tested for *C. difficile* only Category 1 | Pos | 35 |
| | Neg | 48 |
| Tested for *C. difficile* + Other(s) Category 2 | Pos | 22 |
| | Neg | 87 |

## TESTING CATEGORY 1: ONLY TESTING FOR *C. DIFFICILE* WAS REQUESTED

- Among the 406 specimens tested only for *C. difficile* using the Illumigene assay, *C. difficile* was detected in 74 specimens (19.5%).
- 83/406 specimens (35 positive and 48 negative specimens) were retested with the FilmArray GI panel. The FilmArray GI panel detections were 100% concordant with Illumigene®.

**Table 2. Concordance of *C. difficile* Tests**

| | Illumigene Pos | Illumigene Neg |
|---|---|---|
| FilmArray GI Pos | 35 | 0 |
| FilmArray GI Neg | 0 | 48 |

### ADDITIONAL PATHOGENS DETECTED BY MULTI-TARGET FILMARRAY GI TESTING

**Figure 4. Number of Pathogens and Coinfections Detected in Patients Tested Only for *C. difficile***

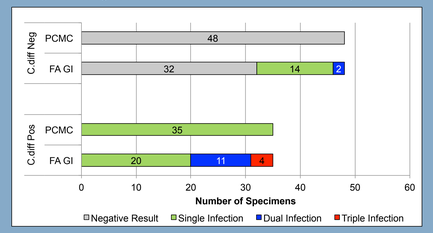



**Table 3. Pathogens Detected in Testing Category 1**

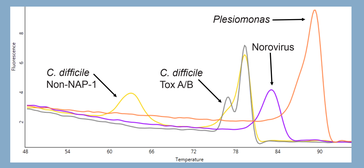
| Coinfections with *C. difficile* | Pathogens detected in *C. difficile* negative specimens |
|---|---|
| Adenovirus F 40/41 | *Aeromonas* |
| ***Campylobacter*** | ***Campylobacter*** |
| EAEC (pAA Plasmid) | **EPEC (eae)** |
| **EPEC (eae +/- bfpA)** | *Giardia lamblia* |
| **Norovirus** | Human Astrovirus |
| *Plesiomonas* | **Norovirus** |
| *Salmonella* | Rotavirus |
| **Sapovirus** | **Sapovirus** |
| | ETEC (*lt/st*) |

Bold indicates a pathogen that was detected in both groups.

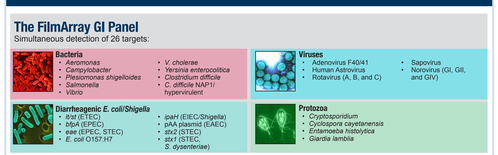
**Figure 5. Sample 0963, an Example of a Polymicrobial Infection as Determined by FilmArray GI from Testing Category 1**
**Sample ID: 0963**

**PCMC Detection**
*C. difficile*

**FilmArray GI Detections**
*C. difficile* (Non-NAP-1)
Norovirus
*Plesiomonas*




## Figure 2. The FilmArray GI Panel




## TESTING CATEGORY 2: TESTING FOR *C. DIFFICILE* WAS REQUESTED IN COMBINATION WITH OTHER TEST(S)

- 327 specimens were tested for *C. difficile* in addition to 1-6 other pathogens as requested by the physician. *C. difficile* was detected in 39 specimens (11.9%).
- 108/ 327 specimens (21 positive and 87 negative for *C. difficile*) were retested with the FilmArray GI panel. The FilmArray GI panel had 98.9% specificity and 95.2% sensitivity compared to the Illumigene.

**Table 4. *C. difficile* Assay Concordance**

| | Illumigene Pos | Illumigene Neg |
|---|---|---|
| FilmArray GI Pos | 20 | 1 |
| FilmArray GI Neg | 1 | 86 |

### ADDITIONAL PATHOGENS DETECTED BY MULTI-TARGET FILMARRAY GI TESTING

**Figure 6. Number of Pathogens and Coinfections Detected in Patients Tested by Standard of Care Versus the FilmArray GI Panel.**

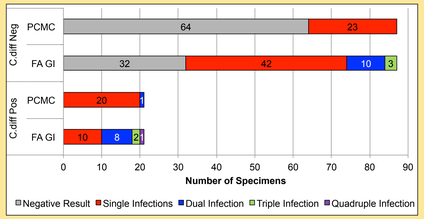



**Table 5. Pathogens Detected in Testing Category 2**

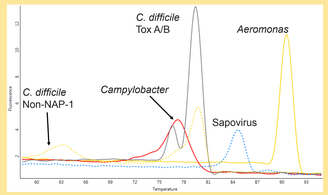
| Coinfections with *C. difficile* | Pathogens detected in *C. difficile* negative specimens |
|---|---|
| **Adenovirus F 40/41** | **Adenovirus F 40/41** |
| ***Aeromonas*** | ***Aeromonas*** |
| ***Campylobacter*** | ***Campylobacter*** |
| **EPEC (eae +/- bfpA)** | EAEC (pAA Plasmid) |
| **Norovirus** | **EPEC (eae +/- bfpA)** |
| ***Salmonella*** | ETEC (*lt/st*) |
| **Sapovirus** | Human Astrovirus |
| | **Norovirus** |
| | ***Salmonella*** |
| | **Sapovirus** |
| | *Shigella*/ EIEC |
| | STEC (eae, *stx1*) |
| | STEC 0157 H7 (*eae*, O157:H7, *stx1, stx2*) |

**Figure 7. Sample 0787, an Example of a Polymicrobial Infection as Determined by FilmArray GI from Testing Category 2**

**Sample ID: 0787**

**PCMC Detection**
*C. difficile*

**FilmArray GI Detections**
*Aeromonas*
*Campylobacter*
*C. difficile* (Non-NAP-1)
Sapovirus




## ACKNOWLEDGEMENTS

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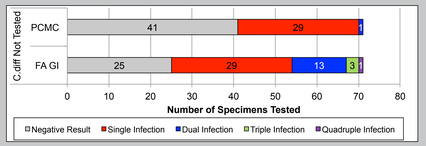
This poster contains information regarding assays that have not been cleared by the FDA for in vitro diagnostic use.

## TESTING CATEGORY 3: *C. DIFFICILE* TESTING WAS NOT REQUESTED

- 299 specimens submitted for other stool tests but not *C. difficile*. Physicians ordered 1-6 tests. A pathogens was identified in 30 (10%) by the clinical lab (one specimen had two pathogens).
- 56/299 specimens (all 30 positive and 41 of the negative) were assessed for 26 pathogens (including *C. difficile*) using the FilmArray GI panel.
- 70 pathogens were detected in 47 specimens with the FilmArray GI panel.
- 6 were (8.5%) were positive for *C. difficile*.

### ADDITIONAL PATHOGENS DETECTED BY MULTI-TARGET FILMARRAY GI TESTING

**Figure 8. Coinfections in Patients Not Tested for *C. difficile***




*FilmArray GI panel detected *C. difficile* in 6 specimens, 3 were coinfected with other pathogen(s).

**Table 6. Pathogens Detected in Testing Category 3**

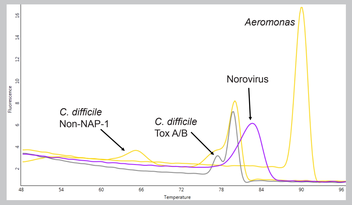
| Pathogens detected specimens not tested for *C. difficile* |
|---|
| Adenovirus F 40/41 |
| *Aeromonas* |
| *Campylobacter* |
| *Clostridium difficile* |
| *Cryptosporidium* |
| EAEC (pAA Plasmid) |
| EPEC (*eae +/- bfpA*) |
| ETEC (*lt/st*) |
| *Giardia lamblia* |
| Human Astrovirus |
| Norovirus |
| Rotavirus |
| *Salmonella* |

**Figure 9. Sample 0377, an Example of a Polymicrobial Infection as Determined by FilmArray GI from the *C. difficile* Not Tested Category**

**Sample ID: 0377**

**PCMC Detection**
*Aeromonas*

**FilmArray GI Detections**
*Aeromonas*
*C. difficile* (Non-NAP-1)
Norovirus

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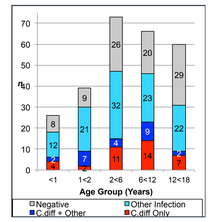
## Patients Tested for *C. difficile* by Age Group

**Table 7.*C. difficile* positive detection rate at the clinical lab by age group.**

| Age | Total # | Pos | % Pos |
|---|---|---|---|
| < 1 | 67 | 17 | 25.4% |
| 1<2 | 98 | 18 | 19.4% |
| 2<6 | 202 | 37 | 18.3% |
| 6<12 | 213 | 35 | 16.4% |
| 12<18 | 158 | 10 | 6.3% |

Among patients diagnosed with *C. difficile* by the clinical lab 23-78% were coinfected with at least one additional pathogen. For those that tested negative for *C. difficile*, other pathogen(s) were detected in 34.9-53.8% of specimens.

**Figure 10. The rate of single *C. difficile* infections and coinfections by age group. A 264 specimen subset from Table 7 was retested with FilmArray GI panel.**




## Table 8. *C. difficile* Positivity Rate by Testing Category

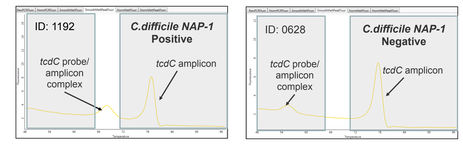
| | Illumigene Results | | | FilmArray GI Results | | |
|---|---|---|---|---|---|---|
| | Total | Pos | Positivity Rate | Total | Pos | Positivity Rate |
| Tested for *C. difficile* only | 407 | 79 | **19.4%** | | | |
| Tested for *C. difficile* + Other(s) | 327 | 39 | **11.9%** | | | |
| Not tested for *C. difficile* | | | | 71 | 6 | **8.5%*** |

*FilmArray GI *C. difficile* assay was 98.2% sensitive and 99.3% specific compared to Illumigene.

## *C. difficile* NAP-1 Detection

- FilmArray GI panel detects deletion in *tcdC* through the melt analysis of an unlabeled probe/ amplicon complex.
- Probe has perfect homology to the *C. difficile NAP-1* strain. Non-NAP-1 strains have mismatches and result in a lower melting temperature of the complex.
- 4 of 47 (8.5%) of *C. difficile* detected by FilmArray GI panel had characteristic deletion in the *tcdC* gene consistent with *NAP-1*/ribotype 27.

**Figure 11. Comparison of *C. difficile NAP-1* Positive and Negative Detections With the FilmArray GI Panel**




## SUMMARY AND CONCLUSIONS

- Performance of FilmArray GI panel for *C. difficile* detection was excellent compared to Illumigene *C. difficile*
- Can potentially identify hypervirulent NAP-1.
- Physician ordering was a poor predictor of CDI. *C. difficile* was detected in 19.4% of patients when *C. difficile* was the only pathogen sought, 11.9% when *C. difficile* and other tests were requested by the clinical lab. CDI was detected by the FilmArray GI panel in 8.5% of specimens where *C. difficile* was not suspected.
- Coinfections with *C. difficile* were common.
  - 43% when only CDI was suspected
  - 52% when CDI or other pathogen(s) were suspected
  - 50% when CDI was not suspected.
- In 33% of patients where only CDI was sought but not detected, the FilmArray GI identified pathogens other than *C. difficile*.
- These data suggest that physician ordering based on clinical presentation and patient history neither accurately predict CDI nor to rule out other infections, supporting the use of a multi-target approach when only CDI is suspected.
- This study justifies the inclusion of test of *C. difficile* in the multi-target FilmArray GI panel.

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