**University of Washington Medical Center**

Clinical Microbiology Laboratory

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| Serology Manual  **Enteric Pathogens by PCR (Biofire) Procedure** |  |

1. Purpose

Acute diarrhea caused by bacterial, viral or parasitic infection represents a significant worldwide healthcare burden. While many cases of diarrhea caused by enteric bacteria, viruses, and parasites are self-resolving and not life-threatening, some pathogens can have serious health implications. Additionally, many gastrointestinal pathogens are of public health concern. The BioFire FilmArray Gastrointestinal (GI) Panel is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with FilmArray systems. The FilmArray GI Panel is capable of the simultaneous detection and identification of nucleic acids from multiple bacteria, viruses, and parasites directly from stool samples in Cary Blair transport media obtained from individuals with signs and/or symptoms of gastrointestinal infection.

The FilmArray GI has been FDA approved to detect and identify the following 22 bacterial, viral, and parasitic targets:

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| --- | --- |
| **Bacteria**  *-Campylobacter (jejuni, coli & upsaliensis)*  *-Clostridium difficile* (Toxin *A/B*)  *-Plesiomonas shigelloides*  *-Salmonella*  *-Yersinia entercolitica*  *-Vibrio (parahaemolyticus, vulnificus & cholerae*)  -*Vibrio cholera* | **Diarrheagenic *E. coli/Shigella***  -Enteroaggregative *E. coli (EAEC)*  -Enteropathogenic *E. coli (EPEC)*  -Enterotoxigenic *E. coli (ETEC) lt/st*  -Shiga-like toxin-producing *E. coli (STEC) stx1/stx2*  *- E. coli* O157  -Shigella/Enteroinvasive *E. coli* (EIEC) |
| **Viruses**  *-*Adenovirus F 40/41  *-*Astrovirus  *-*Norovirus GI/GII  *-*Rotavirus A  *-*Sapovirus (I, II, IV and V) | **Parasites**  *-Cryptosporidium*  -*Cyclospora cayetanensis*  -*Entamoeba histolytica*  -*Giardia lamblia* |

See Section XI for further target information.

**II. Principle**

The FilmArray Gastrointestinal (GI) Panel is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with FilmArray systems. The FilmArray GI Panel is capable of the simultaneous detection and identification of nucleic acids from multiple bacteria, viruses, and parasites.

The FilmArray GI pouch is a disposable closed system that houses all the chemistry required to isolate, amplify and detect nucleic acid from multiple gastrointestinal pathogens within a single stool specimen. The rigid plastic component (fitment) of the FilmArray GI pouch contains reagents in freeze-dried form. The flexible plastic portion of the pouch is divided into discrete segments (blisters) where the required chemical processes are carried out. The user of the FilmArray GI Panel loads the sample into the FilmArray GI pouch, places the pouch into the FilmArray instrument/Module, and starts the run. All other operations are automated.

A. The following is an overview of the operations and processes that occur during a FilmArray run:

1. Nucleic Acid Purification - Nucleic acid purification occurs in the first three blisters of the pouch. The sample is lysed by a combination of chemical and mechanical (bead beating) mechanisms and the liberated nucleic acid is captured, washed and eluted using magnetic bead technology. These steps require about ten minutes, and the bead-beater apparatus can be heard as a high-pitched whine during the first few minutes of operation.

2. Reverse Transcription and 1st Stage Multiplex PCR - Since the GI Panel includes RNA viruses, a reverse transcription (RT) step is performed to convert the viral RNA into cDNA prior to amplification. The purified nucleic acid solution is combined with a preheated master mix to initiate the RT step and subsequent thermocycling for multiplex PCR. The effect of 1st stage PCR is to enrich for the target nucleic acids present in the sample.

3. 2nd Stage PCR - The products of 1st stage PCR are diluted and mixed with fresh PCR reagents containing an intercalating fluorescent DNA dye (LCGreen® Plus, BioFire Diagnostics, LLC). This solution is distributed over the 2nd stage PCR array. The individual wells of the array contain primers for different assays (each present in triplicate) that target specific nucleic acid sequences from each of the pathogens detected, as well as control template material. These primers are ‘nested’ or internal to the specific products of the 1st stage multiplex reaction, which enhances both the sensitivity and specificity of the reactions.

4. DNA Melting Analysis – After 2nd stage PCR, the temperature is slowly increased and fluorescence in each well of the array is monitored and analyzed to generate a melt curve. The temperature at which a specific PCR product melts (melting temperature or Tm) is consistent and predictable and the FilmArray software automatically evaluates the data from replicate wells for each assay to report results. For a description of data interpretation and reporting see the Interpretation of Results section in the Biofire FilmArray Gastrointestinal (GI) Panel Instruction Booklet.

The FilmArray software controls the operation of the instrument/Module, collects and analyzes data, and automatically generates a test report at the end of the run. The entire process takes about an hour.

**III. Specimen**

A. Stool

1. Stool specimens preserved in Cary Blair medium or FecalSwab Cary Blair and within 3 days of collection.
2. Fresh stool collected in a clean container. Fresh stool specimens must be received within two hours of collection and placed into the Cary Blair medium in the laboratory before being tested. Cary Blair preserved specimens may be stored at room temperature, or preferably at 2 - 8°C.
3. Cary Blair received frozen is also acceptable for Biofire testing only. The culture portion will be credited.

Note: A minimum sample volume of 0.2 mL (200μL) of sample is required for testing.

B. Unacceptable specimens

1. Stool submitted on patients hospitalized longer than 3 days. We will perform the test if the provider calls to request testing to be done.
2. Preserved stools in Cary Blair received >3 days after collection.
3. Specimens that are dried out.
4. Specimens that may have leaked out of the container.
5. Inadequately identified specimen – unlabeled, mislabeled, without requisition. (Refer to the UWMC administrative policies and procedure for mislabeled/unlabeled specimens.)
6. Grossly soiled or contaminated container.
7. Inappropriate or insufficient specimen for test requested.
8. Pooled specimen, such as a 24-hour stool collection.
9. The FilmArray GI Panel should not be used for a test of cure. Reject specimens if a patient has had a previous positive in the last 7 days.

|  |  |  |
| --- | --- | --- |
|  | Day shift | Evening shift |
| Specimens: | * Specimen will be placed in BioFire labeled container in Serology | * Setup will tell evening shift MLS stool specimen has arrived * A copy of slip will be place in scope area * Specimen will be placed in BioFire container in set-up refrigerator |
| Run: | * Run as received * Notify evening shift if Biofire is in progress | * Prioritize stool specimens from ED and inpatients * Run as received, time permitting * Specimens received by 21:00 should be tested the same day * Place results on BioFire clipboard |

**IV. Reagents/Supplies**

1. FilmArray GI Panel
2. All kit components should be stored and used together at room temperature (15–25 ºC), including reagent pouches and buffers. Do not use components from one kit with those of another kit. **DO NOT REFRIGERATE.**
3. Avoid storage of any materials near heating or cooling vents or in direct sunlight.
4. Do not remove pouches from their packaging until a sample is ready to be tested. Once the pouch packaging has been opened, the pouch should be loaded as soon as possible (within approximately 30 minutes).
5. Wear gloves, lab coat, and eye protection.

The FilmArray GI Panel consists of the following components:

* Individually packaged FilmArray GI Panel vacuum packed pouches
* Single-use (1.0 mL) Sample Buffer ampoules
* Single-use pre-filled (1.5 mL) Hydration Injection Vials (blue)
* Single-use Sample Injection Vials (red)
* Individually packaged Transfer Pipettes

1. Maine Molecular Pooled External Controls (FilmArray® GI Control Panel M238. Store at -20°C. Thaw for 1 hour prior to use.)
2. Cary Blair Medium for the Detection of Enteric Pathogens (Store at 15 - 35°C)
3. 10% bleach or a similar disinfectant (i.e. Bleach-Rite® disinfecting spray, etc)
4. 70% alcohol solution
5. Disposable gloves

**V. Equipment**

A. FilmArray System

* Biofire Torch
* Printer

1. Pouch Loading Station
2. Biological Safety Cabinet

**VI. Procedure**

Note:

* Never use any Tips, Trays, Tubes, or Test Cartridges which have been broken, cracked, punctured, previously used or anyway visibly damaged; using damaged material may lead to false results.
* Handle supplies, reagents, and kits with powder-free gloves at all times to avoid contamination and change gloves between removal of used disposables and loading of new disposables.
* Handle samples carefully. Open one tube or sample at a time to prevent sample contamination.
* Inadequate or inappropriate specimen collection, storage, or transport may yield false-negative results.

A. Before Preparing Pouch

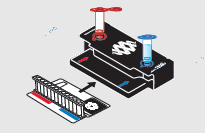
* 1. Put on fresh gloves.
  2. Wipe down the outside of the specimen vial with a lint-free decontaminating wipe.
  3. Thoroughly mix the specimen using a vortex mixer.

B. Prepare Pouch

1. **Perform routine cleaning before each test is run**. Thoroughly clean the work area and the FilmArray Pouch Loading Station with freshly prepared 10% bleach (or suitable disinfectant) followed by a 70% alcohol rinse.
2. Place the following required materials in the clean hood:

* One FilmArray GI Panel pouch
* One Sample Buffer ampoule
* One Hydration Injection Vial (blue cap)
* One Sample Injection Vial (red cap)
* One Transfer Pipette

1. Place the blue-capped Hydration Injection Vial in the blue well of the Pouch Loading Station.
2. Place the red-capped Sample Injection Vial in the red well of the Pouch Loading Station.
3. Obtain patient sample and place into hood.
4. Remove the FilmArray GI pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective aluminum canister.
5. Label the pouch with an accession label.
6. Slide the pouch into the Pouch Loading Station so that the red and blue labels on the pouch align with the red and blue arrows on the Pouch Loading Station.



**Figure 1—Prepare Pouch steps 3 through 8.**

C. Hydrate Pouch

NOTE: If the vacuum seal of the pouch is not intact, the pouch may still be used. Attempt to hydrate the pouch using the following steps. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.

1. Twist the Hydration Injection Vial (blue cap), leaving cap in Pouch Loading Station, and insert the tip of the cannula into the hydration port of the pouch located directly below the blue arrow of the Pouch Loading Station.
2. Push down forcefully in a firm and quick motion until you hear a faint “pop” and feel an ease in resistance. The correct volume of liquid will be pulled into the pouch by vacuum.

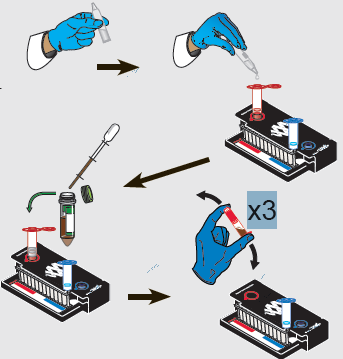


**Figure 2—Hydrate Pouch steps 1 & 2.**

1. Verify that the pouch has been hydrated. Flip the barcode label down and check to see that fluid has entered the reagent wells (located at the base of the rigid plastic part of the pouch). Small air bubbles may be seen. If the pouch fails to hydrate (dry reagents appear as white pellets), verify that the seal of the port was broken by ensuring the vial cannula was fully inserted into the hydration port. If the pouch still fails to hydrate, retrieve a new pouch and repeat from Step 2 of the Prepare Pouch section.

D. Prepare Sample Mix

1. Hold the Sample Buffer ampoule so that the tip is facing up.
2. Gently pinch the textured plastic tab on side of ampoule until the seal snaps.
3. Re-position thumb and forefinger to grip between the textured plastic tab and the bottom of the ampoule, then invert over the red Sample Injection Vial and dispense Sample Buffer using a slow, forceful squeeze, followed by a second squeeze. Avoid generating excessive bubbles.
4. Thoroughly mix the patient specimen.
5. Using the Transfer Pipette provided in the test kit, draw stool sample to the second line (approximately 0.2 mL). Add sample to the red Sample Injection Vial.
6. Tightly close the lid of the Sample Injection Vial and mix by gently inverting at least 3 times.
7. Return the Sample Injection Vial to the Pouch Loading Station.



**Figure 3—Sample Preparation steps 1 through 6.**

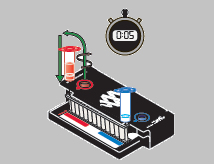
E. Load Sample Mix

1. Slowly unscrew the Sample Injection Vial from the cap and pause for 3-5 seconds. **NOTE: It is important to pause after unscrewing the Sample Injection Vial to avoid sample leakage and contamination of the work area.**

2. Remove Sample Injection Vial leaving cap in Pouch Loading Station and insert the cannula tip into the port in the pouch fitment located directly below the red arrow of the Pouch Loading Station. Push down forcefully in a firm and quick motion until you hear a faint “pop” and feel an ease in resistance. The correct volume of liquid will be pulled into the pouch by vacuum.

3. Verify that the sample has been loaded. Flip the barcode label down and check to see that fluid has entered the reagent well next to the sample loading port. If the pouch fails to pull sample from the Sample Injection Vial, the pouch should be discarded. Retrieve a new pouch and repeat from Step 2 of the Prepare Pouch section.

4. Discard the Hydration Injection Vial and Sample Injection Vial in an appropriate biohazard sharps container.



**Figure 4—Loading the Sample Mix**

F. Run Pouch

The FilmArray Software includes step-by-step on-screen instructions that guide the operator through performing a run.

1. Ensure that the Biofire Torch is on and the touch screen is on ‘Dashboard’.
2. Initiate a run either by Manual Initiation or Scan Initiation.
3. Manual initiation:   
   a. Select an available Torch module from the dashboard using the touch screen.

b. Scan the pouch barcode on the fitment label. Pouch identification (Lot Number and Serial Number), Pouch Type and Protocol are preprogrammed in the barcode located on the FilmArray pouch and will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, Pouch Type and Protocol can be manually entered from the information provided on the pouch label. Then scan or manually enter the sample ID.

c. Insert the pouch into the selected module. The module’s LED will blink blue. Ensure that the pouch fitment label is lying flat on top of the pouch and not folded over. As the pouch is inserted, the module will grab onto the pouch and pull it into the chamber.

1. Scan Initiation:
   1. Scan the pouch barcode on the fitment label. Then scan or manually enter the sample ID.
   2. Insert the pouch into any available module. All available modules’ LED will blink blue. Ensure that the pouch fitment label is lying flat on top of the pouch and not folded over. As the pouch is inserted, the module will grab onto the pouch and pull it into the chamber.
2. After the pouch is correctly inserted into the module, the LED will blink green to indicate that the pouch has been seated, but not yet started.
3. Enter username and password, then select ‘Next’. The ‘Next’ button will only become available when a correct username and password is entered.
4. Review run information on the screen and if correct, select ‘Start Run’
5. Once the run has been started, the module’s LED will turn solid green, indicating the run is in progress. The display also changes to the ‘Run in Progress’ screen and shows the steps that the module is currently performing and the approximate remaining run time.
6. At the end of the run, the Dashboard changes to the status of the module to Finished and the pouch is partially ejected. The report will print out automatically. Note: Once the pouch has been removed, the report can only be viewed through the Browse Runs feature.
7. Immediately discard the pouch in a biohazard container.
8. Save Cary Blair in labeled box located in the refrigerator behind the BC2 bench. Toss after 7 days.
9. **WARNING: If liquid is observed on the exterior of a pouch after removal, the liquid and pouch should be immediately contained and discarded in a biohazard container. The instrument/Module and work space must be decontaminated as described in section X. Maintenance. DO NOT PERFORM ADDITIONAL TESTING UNTIL THE AREA HAS BEEN DECONTAMINATED.**

**VII. Interpretation**

A. Assay Interpretation

When 2nd stage PCR is complete, the FilmArray instrument/Module performs a high resolution DNA melting analysis on the PCR products and measures the fluorescence signal generated in each well. The FilmArray software then performs several analyses and assigns a final assay result. The steps in the analysis are described below.

1. **Analysis of melt curves**—The FilmArray software evaluates the DNA melt curve for each well of the 2nd stage PCR array to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature (Tm) of the curve. The Tm value is then compared against the expected Tm range for the assay. If the software determines that the melt curve is positive and the Tm falls inside the assay-specific Tm range, the melt curve is called positive. If the software determines that the melt curve is negative or is not in the appropriate Tm range, the melt curve is called negative.
2. **Analysis of replicates**—Once melt curves have been identified, the software evaluates the three replicates for each assay to determine the assay result. For an assay to be called positive, at least two of the three associated melt curves must be called positive, and the Tm for at least two of the three positive melt curves must be similar (within 1°C). Assays that do not meet these criteria are called negative.

B. Film Array GI Test Report

The FilmArray GI test report is automatically displayed upon completion of a run and contains three sections, the Run Summary, the Result Summary, and the Run Details. The test report can be saved as a PDF or printed.

The **Run Summary** section of the test report provides the Sample ID, time and date of the run, control results and an overall summary of the test results. Any organism with a Detected result will be listed in the corresponding field of the summary. If all of the tests were negative then None will be displayed in the Detected field. Controls are listed as Passed, Failed or Invalid. See the Controls Field section below for detailed information about the interpretation of controls and appropriate follow-up in the case of control failures.

The **Result Summary** section of the test report lists the result for each target tested by the panel. Possible results for each organism are Detected, Not Detected, Not Applicable (N/A), or Invalid. See Results Summary section below for detailed information about interpretation of test results and appropriate follow-up for Invalid results.

The **Run Details** section provides additional information about the run including: pouch information (type, lot number, and serial number), Run Status (Completed, Incomplete, Aborted, Instrument Error, Instrument Communication Error, or Software Error), the protocol that was used to perform the test, the identity of the operator that performed the test, and the instrument/Module used to perform the test.

Once a run has completed, it is possible to edit the Sample ID. If this information has been changed, an additional section called **Change History** will be added to the test report. This Change History section lists the field that was changed, the original entry, the revised entry, the operator that made the change, and the date that the change was made. Sample ID is the only field of the report that can be changed.

C. Controls Field

The Controls field on the test report will display Passed, Failed, or Invalid. The Controls field will display Passed only if the run completed successfully (no instrument or software errors) and both of the pouch control assays (RNA Process Control and PCR2 Control) were successful. The Controls field will display Failed if the run was completed successfully (no instrument or software errors) but one or both of the pouch control assays failed. If the control result is Failed, then the result for all of the tests on the panel are displayed as Invalid and the sample will need to be retested with a new pouch. Table 1 provides a summary and explanation of the possible control results and follow-up actions.

**Table 1—Interpretation of Controls Field on the FilmArray GI Test Report**

|  |  |  |  |
| --- | --- | --- | --- |
| **Control Result** | **Explanation** | **Action Required** | **Outcome** |
| Passed | The run was successfully completed  AND  Both pouch controls were successful. | None | Report the results provided on the test report. |
| Failed | The run was successfully completed  BUT  At least one of the pouch controls (RNA Process Control and/or PCR2 Control failed. | Repeat the test using a new pouch. | Accept the results of the repeat testing. If the error persists, contact Technical Support for further instruction. |
| Invalid | The controls are invalid because the run did not complete.  (Typically this indicates a software or hardware error). | Note any error codes displayed during the run and the Run Status field in the Run Details section of the report. Refer to the FilmArray Operator’s Manual or contact Technical Support for further instruction.  Once the error is resolved, repeat the test or repeat the test using another instrument.  If the error occurred in the first 30 seconds of the run, the same pouch may be used for the repeat test (within 60 minutes of pouch loading) using the same instrument or another instrument, as available.  If the error occurred later in the run or you are unsure when the error occurred, return to the original sample to load a new pouch. Repeat the test with the new pouch on the same instrument or another instrument, as available. | Accept the valid results of the repeat testing. If the error persists, contact Technical Support for further instruction. |

D. Results Summary – Interpretations

The Results Summary – Interpretations section provides a complete list of the test results. Possible results for each organism include Detected, Not Detected, N/A and Invalid. Table 2 provides an explanation for each interpretation and any follow-up necessary to obtain a final result.

**Table 2— Results Summary – Interpretations**

|  |  |  |
| --- | --- | --- |
| **Result** | **Explanation** | **Action** |
| Detected | The run was successfully completed  AND  The pouch controls were successful (Passed)  AND  The assay(s) associated with the interpretation were positive based on the following requirements for at least 2 of the 3 assay replicates:  -a positive melt curve, and  -the Tm for the melt data were within the assay specific limits, and  -the Tm for the melt data were within 1°C of each other. | None. Report all Positive (detected) results. |

|  |  |  |
| --- | --- | --- |
| Not Detected | The run was successfully completed  AND  The pouch controls were successful (Passed)  AND  The assay(s) associated with the interpretation were negative (did not meet the requirements for a positive assay described in Detected). | None. Report results – not detected (NOEPG) |
| N/A  (applies to *E. coli* O157 and EPEC only) | The run was successfully completed  AND  The pouch controls were successful (Passed)  AND  For *E.* coli O157: Shiga-like toxin-producing *E. coli* was Not Detected. a  For EPEC: Shiga-like toxin producing *E. coli* was Detected.b | None. Report results. For target with N/A consider as not detected. |
| Invalid | The run did not complete successfully (Aborted, Incomplete, Instrument Communication Error, Instrument Error, or Software Error)  OR  The pouch controls were not successful (Failed) | See Table 1, *Interpretation of*  *Controls Field on FilmArray*  *Report,* for instruction. |

a *E. coli* O157 result is not applicable when STEC is not detected.

b When a Shiga-like toxin producing *E. coli* is “Detected” the EPEC result is not applicable as its detection cannot be differentiated from *eae* containing STEC.

E. Organism Interpretation

For many organisms detected by the FilmArray GI Panel, the organism is considered to be “Detected” if a single corresponding assay is positive. For example, *Plesiomonas shigelloides* will have a result of “*Plesiomonas shigelloides* Detected” if at least two of the three replicates of the one *Plesiomonas shigelloides* assay have similar positive melt peaks with Tm values that are within the assay-specific Tm range.

The following organisms are detected using a single assay: toxigenic *C. difficile*, *P. shigelloides, Salmonella, Y. enterocolitica*, EAEC, *Shigella/*EIEC, Adenovirus F 40/41, Astrovirus, Sapovirus (Genogroups I, II, IV, and V), *C. cayetanensis, E. histolytica* and *G. lamblia*.

In contrast, the test results for several organisms rely on the combination of multiple assays. These include *Campylobacter (C. jejuni/C. coli/C. upsaliensis), Vibrio* (*V. parahaemolyticus/ V. vulnificus/V. cholerae)* and *Vibrio* *cholerae*, *Cryptosporidium,* Norovirus GI/GII, and Rotavirus A. The test results for several Diarrheagenic *E. coli*(s) include multiple assays for genetic markers to identify various classic pathotypes of *E. coli* including EPEC, ETEC, and STEC (including O157), (as well EAEC and *Shigella*/EIEC included above).

Table 3 below lists the possible test results generated by the BioFire FilmArray GI Panel, representing identification of bacterial, parasitic, viral, and/or genetic virulence marker nucleic acid sequences/ targets.

**VIII. Reporting**

A. BioFire Results:

1. Direct Exam: Report Shiga toxin (STEC) result. If STEC is positive by Biofire, report positive (POS) under the direct exam and report the positive STEC group code (STECPG) under the culture result.
2. Culture Report: Report all positive BioFire targets using the Group Codes found in Table 3. If no targets were detected, enter as ‘no targets detected’ (NOEPG). Enter the corresponding LIS Workup Result code at the STLPCR prompt.
3. If required, call King County Public Health Lab according to the Reportable and Notifiable conditions procedure and Table 3.
4. Call the ordering physician, infection control, or clinic with a positive result according to Table 3.
5. Move positive Cary Blair to labeled box in the refrigerator behind the BC2 bench.
6. Reporting Notes:
7. **Do not report a positive *Vibrio cholerae* result before consulting the Micro Fellow or Director. False positive *Vibrio cholerae* results have been observed**.
8. Report all positive targets. If four or more distinct organisms are detected in a specimen, please review recent positives to look for possible contamination issues. If there is anything unusual about the result, please contact the Micro Fellow.
9. If both STEC (Shiga-like toxin producing *E. coli*) and EIEC (*Shigella*/Enteroinvasive *E. coli*) targets are both detected – consult Micro Fellow.
10. If the Biofire is positive for C. difficile on a patient under 2 years old, report the results and notify the Micro fellow to contact the physician regarding the results.

B Stool Culture Results:

1. Report *Aeromonas* results (Enter a preliminary result on day one)
2. Report either reduced or no normal fecal flora if appropriate
3. Report the following if predominant:

*Staphylocccus aureus*

*Pseudomonas* spp.

*Yeast*

*Bacillus* (Rule out *B. cereus -* consult at Bench rounds before reporting)

1. **All other stool culture results are recorded in workup only.**
2. If susceptibilities are needed, report the organism code for the positive target (do not use the Group Code identification) on a different line than the positive target and enter/accept the susceptibilities.
3. If any discrepancies are found between BioFire results and culture results, consult with Serology Lead.

**Table 3—Reporting Group Codes and Notification Chart**

**Use the following chart for reporting results.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Organism** | **Target**  **Gene** | **Test Result**  **Reported as**  **“Detected”** | **Report Test Result** | | | | **Submit to State Lab** |
| **Group**  **Code** | **LIS Group Code Translation** | **LIS Workup**  **Result Code** | **Critical Result/ Notifiable Condition** |
| Not detected:  Shiga-like toxin-producing  *E. coli* (STEC) *stx1/stx2* | | | ST12NP | Shiga toxins 1 and 2 result: Not detected. Tested by multiplex PCR | N/A | NO | N/A |
| All Analytes - “Not Detected” | | | NOEPG comprised of NOEP1-STLCOM | No gastrointestinal pathogens detected by PCR (Methodology: Multiplex PCR) | BFGPN | NO | N/A |
| *Campylobacter Group*a |  | *Campylobacter* | CAMPPG comprised of CAMP-MPCR-REPT0-ICCOME | Campylobacter PCR test positive (Methodology: Multiplex PCR) - This result is a Washington state Notifiable condition. Contact public health authorities in accordance with WAC 246-101.  http://www.doh.wa.gov/Portals/1/Documents/5100/210-001-Poster-HCP.pdf - For inpatients, isolate using contact enteric precautions per institutional policy. Contact Infection Control if you have any questions. | BFGP1 | YES  Notify Provider | NO |
| *Clostridium difficile*  toxin A/Bb | *tcdA, tcdB* | *Clostridium difficile*  toxin A/B | CDABPG comprised of CDIFF-TOXAB-MPCR - ICCOME | Clostridium difficile toxin A/B PCR test positive (Methodology: Multiplex PCR) - For inpatients, isolate using contact enteric precautions per institutional policy. Contact Infection Control if you have any questions. | BFGP2 | YES  Notify provider (in house patients only) | N/A |
| *Plesiomonas shigelloides* |  | *Plesiomonas shigelloides* | PSHIG comprised of PSHI-MPCR | Plesiomonas shigelloides PCR test positive (Methodology: Multiplex PCR) | BFGP3 | YES  Notify Provider | NO |
| *Salmonella species*c |  | *Salmonella* | SALMPG comprised of SALM- MPCR -REPT0-ICCOME | Salmonella PCR test positive (Methodology: Multiplex PCR)  - This result is a Washington state Notifiable condition. Contact public health authorities in accordance with WAC 246-101.  http://www.doh.wa.gov/Portals/1/Documents/5100/210-001-Poster-HCP.pdf- For inpatients, isolate using contact enteric precautions per institutional policy. Contact Infection Control if you have any questions. | BFGP4 | YES  Notify Provider | Yes  Isolate  If culture negative, send Cary Blair |
| *Vibrio Group*d |  | *Vibrio* | VIBRPG comprised of VIBN- MPCR REPT0-ICCOME | Vibrio spp, not V. cholerae PCR test positive (Methodology: Multiplex PCR)  - This result is a Washington state  Notifiable condition. Contact public health authorities in accordance with WAC 246-101.  http://www.doh.wa.gov/Portals/1/Documents/5100/210-001-Poster-HCP.pdf- For inpatients, isolate using contact enteric precautions per institutional policy. Contact Infection Control if you have any questions. | BFGP5 | YES  Notify Provider | Yes  Isolate |
| *Vibrio cholerae*e  (see footnote) | *toxR* | *Vibrio cholerae*e  (see footnote) | VCHOPG comprised of VCHO- MPCR -REPT0-ICCOME | Vibrio cholerae PCR test positive (Methodology: Multiplex PCR) - This result is a Washington state Notifiable condition. Contact public health authorities in accordance with WAC 246 101. http://www.doh.wa.gov/Portals/1/Documents/5100/210-001-Poster-HCP.pdf - For inpatients, isolate using contact enteric precautions per institutional policy. Contact Infection Control if you have any questions. | BFGP6 | YES  Notify provider, but  consult with Micro Fellow or Director before reporting.e  Call immediately to live person at Public Health Lab\* | YES  Give isolate to BT MLS |
| *Yersinia enterocolitica* |  | *Yersinia enterocolitica* | YENTPG comprised of YENT- MPCR -REPT0-ICCOME | Yersinia enterocolitica PCR test positive (Methodology: Multiplex PCR) - This result is a Washington state Notifiable condition. Contact public health authorities in accordance with WAC 246-101.  http://www.doh.wa.gov/Portals/1/Documents/5100/210-001-Poster-HCP.pdf- For inpatients, isolate using contact enteric precautions per institutional policy. Contact Infection Control if you have any questions. | BFGP7 | YES  Notify Provider | NO |
| Enteroaggregative  *E. coli* (EAEC) | *aggR, aatA* | Enteroaggregative  *E. coli* (EAEC) | EAECPG comprised of EAECP-MPCR | Diarrheagenic Enteroaggregative E. coli (EAEC) PCR test positive (Methodology: Multiplex PCR) | BFGP8 | NO | NO |
| Enteropathogenic  *E. coli* (EPEC) |  | Enteropathogenic  *E. coli* (EPEC) | EPECPG comprised of EPECP-MPCR | Diarrheagenic Enteropathogenic E. coli (EPEC) PCR test positive (Methodology: Multiplex PCR) | BFGP9 | NO | NO |
| Enterotoxigenic  *E. coli* (ETEC) *lt/st* | *itA, st1a, st1b* | Enterotoxigenic  *E. coli* (ETEC) *lt/st* | ETECPG comprised of ETECP-MPCR | Diarrheagenic Enterotoxigenic E. coli (ETEC) PCR test positive (Methodology: Multiplex PCR) | BFGP10 | NO | NO |
| Shiga-like toxin-producing  *E. coli* (STEC) *stx1/stx2*f | *stx1, stx2* | Shiga-like toxin-producing  *E. coli* (STEC) *stx1/stx2* | STECPG comprised of STECP1-REPT0-ICCOME | Shiga toxin-producing E. coli (STEC) stx1/stx2, Not O157, PCR test positive (Methodology: Multiplex PCR)   -  This result is a Washington state Notifiable condition. Contact public health authorities in accordance with WAC  246-101. http://www.doh.wa.gov/Portals/1/Documents/5100/210-001-Poster-HCP.pdf - For inpatients, isolate using contact enteric precautions per institutional policy. Contact Infection Control if you have any questions. | BFGP11 | YES  Notify provider  Call immediately to live person at Public Health Lab\* | YES  Cary Blair |
| *E. coli* O157g |  | *E. coli* O157 | O157PG comprised of O157P1-REPT0-ICCOME | Shiga toxin-producing E. coli (STEC) stx1/stx2, O157 serotype PCR test positive (Methodology: Multiplex PCR) -  This result is a Washington state Notifiable condition. Contact public health authorities in accordance with WAC  246-101. http://www.doh.wa.gov/Portals/1/Documents/5100/210-001-Poster-HCP.pdf. -For inpatients, isolate using contact enteric precautions per institutional policy. Contact Infection Control if you have any questions. | BFGP12 | YES  Notify provider  Notify Micro Fellow | Yes  Cary Blair |
| *Shigella/*Enteroinvasive *E. coli* (EIEC)f, h | *ipaH, stx* | *Shigella/*Enteroinvasive *E. coli* (EIEC) | SHIGPG comprised of SHIG-EIEC- MPCR REPT0-ICCOME | Shigella or Enteroinvasive E. coli (EIEC) PCR test positive (Methodology: Multiplex PCR) - This result is a   Washington state Notifiable condition. Contact public health authorities in accordance with WAC 246-101.  http://www.doh.wa.gov/Portals/1/Documents/5100/210-001-Poster-HCP.pdf- For inpatients, isolate using contact enteric precautions per institutional policy. Contact Infection Control if you have any questions. | BFGP13 | YES  Notify Provider | Yes  Isolate |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Organism** | **Target**  **Gene** | **Test Result**  **Reported as**  **“Detected”** | **Report Test Result** | | | | **Submit to State Lab** |
| **Group**  **Code** | **LIS Group Code Translation** | **LIS Workup**  **Result Code** | **Critical Result/ Notifiable Condition** |
| *Cryptosporidium* |  | *Cryptosporidium* | CRYPG comprised of CRYPT1- MPCR -REPT0 | Cryptosporidium PCR test positive (Methodology: Multiplex PCR) - This result is a Washington state Notifiable condition. Contact public health authorities in accordance with WAC 246-101. http://www.doh.wa.gov/Portals/1/Documents/5100/210-001-Poster-HCP.pdf | BFGP14 | YES  Notify Provider | N/A |
| *Cyclospora cayetanensis* |  | *Cyclospora cayetanensis* | CYCLPG comprised of CCAY- MPCR -REPT0 | Cyclospora cayetanensis PCR test positive (Methodology: Multiplex PCR) - This result is a Washington state Notifiable condition. Contact public health authorities in accordance with WAC 246-101. http://www.doh.wa.gov/Portals/1/Documents/5100/210-001-Poster-HCP.pdf | BFGP15 | YES  Notify Provider | Yes  Cary Blair |
| *Entamoeba histolytica* |  | *Entamoeba histolytica* | EHISPG comprised of EHIS- MPCR | Entamoeba histolytica PCR test positive (Methodology: Multiplex PCR) | BFGP16 | YES  Notify Provider | N/A |
| *Giardia lamblia* |  | *Giardia lamblia* | GLAMPG comprised of GIAR1- MPCR REPT0 | Giardia lamblia PCR test positive (Methodology: Multiplex PCR) - This result is a Washington state Notifiable condition. Contact public health authorities in accordance with WAC 246-101.  http://www.doh.wa.gov/Portals/1/Documents/5100/210-001-Poster-HCP.pdf | BFGP17 | YES  Notify Provider | N/A |
| Adenovirus F 40/41 |  | Adenovirus F 40/41 | ADENPG comprised of ADENP- MPCR ICCOME | Adenovirus PCR test positive (Methodology: Multiplex PCR) - For inpatients, isolate using contact enteric precautions per institutional policy. Contact Infection Control if you have any questions. | BFGP18 | NO | N/A |
| Astrovirus |  | Astrovirus | ASTRPG comprised of ASTRP- MPCR | Astrovirus PCR test positive (Methodology: Multiplex PCR) | BFGP19 | NO | N/A |
| Norovirus GI/GII |  | Norovirus | NOROPG comprised of NOROP1- MPCR -ICCOME | Norovirus PCR test positive (Methodology: Multiplex PCR) - For inpatients, isolate using contact enteric precautions per institutional policy. Contact Infection Control if you have any questions. | BFGP20 | NO | N/A |
| Rotavirus A |  | Rotavirus | ROTAPG comprised of ROTAP1- MPCR ICCOME | Rotavirus PCR test positive (Methodology: Multiplex PCR) - For inpatients, isolate using contact enteric precautions per institutional policy. Contact Infection Control if you have any questions. | BFGP21 | NO | N/A |
| Sapovirus |  | Sapovirus | SAPOPG comprised of SAPOP- MPCR | Sapovirus PCR test positive (Methodology: Multiplex PCR) | BFGP22 | NO | N/A |

a *C. coli, C. jejuni,* and *C. upsaliensis*

b If positive on a patient under 2 years old, report the results and notify the Micro fellow to contact the physician regarding the results.

c *S.enterica, S. bongori*

d *V .parahaemolyticus, V. vulnificus, V. cholera.* Empirical testing and in silico sequence analysis indicate that the assay may also react with some less common *Vibrio* species(i.e., *V. alginolyticus, V. fluvialis,* and *V. mimicus).*

e Do not report a positive *Vibrio cholerae* result before consulting the Micro Fellow or Director. False positive *Vibrio cholerae* results have been observed.

f Shiga toxin (*stx*; identical to *stx1* of STEC) is found in *Shigella dysenteriae*, therefore a FilmArray GI Panel report with positive test results for Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2* with *Shigella*/Enteroinvasive *E. coli* (EIEC) in the same sample may indicate the presence of *S. dysenteriae*. Consult the Micro fellow.

g O157 determinant may be from the STEC or may be due to the rare possibility of a shiga-like toxin-negative *E. coli* O157 being in the same specimen with a non-O157 STEC.

h The FilmArray GI Panel contains a single assay (Shig) for the detection of *ipaH,* a gene specifically found in all *Shigella* species as well as Enteroinvasive *E. coli* (EIEC). It is not possible to differentiate *Shigella* from EIEC using this method, and detection of *ipaH* will result in a *Shigella*/Enteroinvasive *E. coli* (EIEC) Detected test result.

\*Immediately Notifiable Condition: 206-296-4774 or 206-296-4782. After hours: 206-726-2128

C. Printouts

1. Day shift: Place print-out of FilmArray GI Panel results in BioFire study notebook

2. Evening shift: Place print-out of FilmArray GI panel on clipboard for dayshift to review or call

|  |  |  |
| --- | --- | --- |
|  | Day shift | Evening shift |
| Reporting  AND  Call | * Report or call all previous evening positive results according to Table 3. Results from this shift can be found on the BioFire clipboard. * Report or call all positive results from the day shift according to Table 3. * Call *Aeromonas* positives from stool culture * Call positive *C. difficile* (inpatient only) * Call Infection Control (inpatient only) | * Report and call positive results for all inpatients (See Table 3). Clinic patient results will be called by dayshift. Place all printouts of BioFire results on the clipboard in Serology * Call all STEC * Call all *Vibrio cholerae* (after confirmed as not a false positive, see Section VIII. Reporting for details) * Call positive *C. difficile* (inpatient only) * Call Infection Control (inpatient only) |
| Reporting | Positive BioFire targets that are reported but NOT called are: *Pleisiomonas*, EAEC, EPEC, ETEC, Norovirus, Rotavirus, Adenovirus, Astrovirus, Sapovirus, Positive *C. difficile* (outpatient only) | Positive BioFire targets that are reported but NOT called include: *Pleisiomonas*, EAEC, EPEC, ETEC, Norovirus, Rotavirus, Adenovirus, Astrovirus, Sapovirus, Positive *C. difficile* (outpatient only) |

**IX. Quality Control**

New lot number/shipment and Monthly Testing QC will be performed by the Serology Bench MLS. See ‘Procedure for Serology Quality Control Guidelines’, document #616.U.101.xx found in the Serology Manual.

A. Initial Quality Control

External quality control is performed on each new lot number/ shipment prior to patient testing. Testing includes the Maine Molecular (MMQCI) FilmArray GI Control Panel M238 and a negative control. The Maine Molecular GI Panel M238 is comprised of synthetic RNA suspended in a non-infectious solution of buffers, preservatives and stabilizers. The MMQCI Panel contains vials of two pre-mixed, color-coded pools of bacterial/parasitic/viral targets as described in Table 4 below:

**Table 4—Maine Molecular FilmArray GI Control Panel M238**

|  |  |  |
| --- | --- | --- |
| Assay | QC Panel M239 (Orange) | QC panel M240 (Purple) |
| Bacteria |  |  |
| *Campylobacter* | Not Detected | Detected |
| *Clostridium difficile* toxin A/B | Detected | Not Detected |
| *Plesiomonas shigelloides* | Detected | Not Detected |
| *Salmonella* | Not Detected | Detected |
| *Vibrio* | Detected | Not Detected |
| *Vibrio cholerae* | Detected | Not Detected |
| *Yersinia enterocolitica* | Detected | Not Detected |
| Diarrheagenic E. coli/Shigella |  |  |
| Enteroaggregative *E. coli* (EAEC) | Detected | Not Detected |
| Enteropathogenic *E. coli* (EPEC) | Ø N/A | Detected |
| Enterotoxigenic *E. coli* (ETEC) *lt/st* | Not Detected | Detected |
| Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2* | Detected | Not Detected |
| *E. coli* O157 | Detected | Ø N/A |
| *Shigella/*Enteroinvasive *E. coli* | Detected | Not Detected |
| Parasites |  |  |
| *Cryptosporidium* | Detected | Not Detected |
| *Cyclospora cayetanensis* | Not Detected | Detected |
| *Entamoeba histolytica* | Not Detected | Detected |
| *Giardia lamblia* | Not Detected | Detected |
| Viruses |  |  |
| Adenovirus F 40/41 | Detected | Not Detected |
| Astrovirus | Not Detected | Detected |
| Norovirus GI/GII | Not Detected | Detected |
| Rotavirus A | Not Detected | Detected |
| Sapovirus | Detected | Not Detected |

Testing the control vials:

1. Allow the control vial to be tested to come to room temperature (18° – 25°C).
2. Use the control as provided.  ***DO NOT DILUTE.***
3. Prepare and hydrate a pouch according to the instructions listed in the **Procedure** section.
4. Immediately before use, mix the control by briefly vortexing the tube for 3 – 5 seconds and then shake the tube down firmly to remove any droplets caught in the cap.
5. Using the FilmArray transfer pipette, draw up the control to **2nd** line and add the control to the FilmArray® Sample Buffer as you would a patient, according to the instructions listed in the **Procedure** section.
6. Load the pouch onto the BioFire FilmArray instrument, identifying the orange M239 FilmArray GI Control Panel, the purple M240 FilmArray GI Control Panel, and negative control.
7. Acceptable results are listed in table 4 above.

Document the external quality control results on the BioFire GI Panel QC Sheet. Print the results and file in the Serology Quality Control Manual.

B. Monthly Quality Control:

Twenty days of quality control testing is required to determine the acceptability of using the internal quality control on a daily basis. Because the twenty days of testing proved acceptable, external quality control is now performed at least monthly (by the 10th of the month) on the in-use lot number of product. This is accomplished by using the Maine Molecular (MMQCI) FilmArray GI Control Panel vials M239 and M240 (see Table 4 for the composition of the control panels and the acceptable results) and a negative control. Document the quality control results on the BioFire FilmArray Quality Control form.

Record the results of the external QC on the Biofire GI Panel QC sheet. Print the results and file into the Serology Quality Control Manual.

C. Quality Control After Major Maintenance or Service

The performance of the instrument/module is verified after major maintenance or service to ensure they are running as expected. Run one positive Maine Molecular FilmArray GI Control Panel (M239 **or** M240) and one negative control. Document the quality control results on the BioFire FilmArray Quality Control form.

D. Internal Control

Two internal controls are included in each pouch:

1. **RNA Process** Control—The RNA Process Control assay targets an RNA transcript from the yeast *Schizosaccharomyces pombe*. The yeast is present in the pouch in a freeze-dried form and becomes rehydrated when sample is loaded. The control material is carried through all stages of the test process, including lysis, nucleic acid purification, reverse transcription, 1st stage PCR, dilution, 2nd stage PCR and DNA melting. A positive control result indicates that all steps carried out in the FilmArray GI pouch were successful.
2. **PCR2** Control—The PCR2 Control assay detects a DNA target that is dried into wells of the array along with the corresponding primers. A positive result indicates that 2nd stage PCR was successful.

Both control assays must be positive for the test run to pass. If either control fails, the Controls field of the test report (upper right hand corner) will display “Failed” and all results will be listed as Invalid. If the controls fail, the sample should be retested using a new pouch. The FilmArray software will automatically fail the run if the melting temperature (Tm) for either the RNA Process Control or the PCR2 Control is outside an acceptable range (80.2-84.2 for the RNA Process Control and 74.1-78.1 for the PCR2 Control).

**X. Maintenance**

Mark each item as completed on the BioFire Maintenance Log.

Note: **10% bleach and 70% ethanol cleaning is critically important to avoid DNA contamination and false-positive results.**

A. Daily

1. Bench Cleaning—Around the BioFire instrument
   1. Wipe bench with 10% bleach disinfectant (e.g., 10% bleach solution, Bleach-Rite® disinfecting spray, etc.).
   2. Wipe bench with 70% ethanol.

2. Instrument Cleaning

a. Wipe the outside of the BioFire instrument with 10% bleach disinfectant. To wipe the touch screen, disable the screen by selecting ‘Settings’ and then select ‘Switch to Cleaning Mode’. The touch screen is inactivated for 30 seconds.

b. Wipe the outside of the BioFire instrument with 70% ethanol.

c. Do not remove the module front cover and clean underneath it, except in the case of a contamination event.

3. Pouch Loading Station Cleaning

a. Wipe the Pouch Loading Station down with 10% bleach disinfectant.

b. Wipe the Pouch Loading Station with 70% ethanol.

4. Biological Safety Cabinet Cleaning – Prior to the first morning run and if there is a spill, clean the Biological Safety Cabinet (BSC):

a. Remove items from the immediate work surface.

b. Spray/ squirt 10% bleach disinfectant on work surface.

c. Wipe the 10% bleach disinfectant on BSC work surface with paper towels and discard.

d. Spay/ squirt 70% ethanol on work surface.

e. Wipe with paper towels and discard.

B. Weekly (Perform each Thursday)

* 1. Decontamination of the Pouch Loading Station

a. Submerge the Pouch Loading Station in a tub containing 10% bleach solution.

b. Soak the Pouch Loading Station for 15 minutes.

c. Remove the Pouch Loading station from the bleach.

d. Rinse 3 different times using fresh dH20

1. Wipe with paper towels and discard.

2. Clean the lens of the barcode reader using lens cleaner.

3. Check ink printer levels

4. Restart computer. Ensure all applications are closed before attempting to restart the computer.

C. Decontamination in the Case of Pouch Leakage

1. Dispose of leaking pouch in a biohazard container as well as potentially contaminated gloves and lab coat. Replace with clean PPE.

2. Wet a paper towel with 10% bleach disinfectant and wipe all exterior surfaces of the Torch, including the screen (in cleaning mode). Repeat twice with fresh paper towels for a total of 3 bleach wipes.

3. Change gloves, then wet a paper towel with 70% ethanol and wipe all exterior surfaces of the Torch. Repeat once with a fresh paper towel.

4. Remove the module front cover. Repeat steps 2 through 3 for inner front cover surfaces and pouch slots.

**XI. Target Information**

*Campylobacter* (*C. jejuni/C. coli/C. upsaliensis*)—*Campylobacter* infection is the most common cause of bacterial gastroenteritis. Antibiotics are generally not prescribed unless symptoms are severe. Delaying treatment for several days while waiting for culture methods to confirm the presence of *C. jejuni*, can reduce the effectiveness of the therapy.

*Plesiomonas shigelloides.—Plesiomonas shigelloides*, Gram-negative rod-shaped bacteria and members of the *Enterobacteriaceae* family, are isolated from a wide range of environmental sources including freshwater and many wild and domestic animals. *P. shigelloides* gastroenteritis can occur from the consumption of contaminated seafood or water. Symptoms generally include watery diarrhea, though dysenteric diarrhea can occur, and infections may be prolonged (>2 weeks duration) but are generally self-limiting. Most cases reported in the US are from individuals with pre-existing health problems leading to a more severe disease outcome.

*Salmonella* spp.—Infection with non-typhoidal *Salmonella* spp. causes diarrhea and fever. Approximately one million cases are known to occur annually in the U.S. Most cases of *Salmonella* infection are typically self-resolving. However, children, elderly, and immunocompromised patients may require therapy to resolve symptoms. Antimicrobial therapy can prolong the duration of non-typhoidal *Salmonella* and is only recommended for patients with severe symptoms.

*Yersinia enterocolitica*—*Y. enterocolitica* infections can resemble Crohn’s disease or appendicitis, with symptoms including diarrhea and fever. There is an estimated one confirmed case of *Y. enterocolitica* infection per 100,000 people in the U.S. each year. These infections are self-limiting and do not generally require antibiotics.

*Vibrio* spp.—*Vibrio* spp. infections are acquired by the consumption of contaminated water or raw or undercooked shellfish, especially oysters. *V. parahaemolyticus* is responsible for approximately 4,500 cases of gastrointestinal illness in the United States annually. *V. parahaemolyticus* infections typically cause watery diarrhea along with nausea, vomiting, and abdominal cramps. Treatment is not usually required in a majority of cases. *V. cholerae* infections result in a profuse, watery diarrhea and vomiting. *V. cholerae* is relatively uncommon in developed countries, causing less than 100 cases annually in the U.S. Antibiotics can be used to shorten the duration of symptoms associated with *V. cholerae* infection. *V. vulnificus* can cause gastrointestinal illness and some patients develop bullous skin lesions.

*Shigella* spp.—Nearly 14,000 cases of infection with *Shigella* are reported each year in the United States. Patients infected with *Shigella* often develop fever, stomach cramps, and bloody diarrhea. Although patients with mild *Shigella* infections usually recover without antibiotic treatment, antibiotics may be used to treat severe cases of *Shigella* infection. Antidiarrheal agents can worsen illness and should be avoided.

Shiga toxins 1 and 2—Shiga toxin-producing *Escherichia coli* (STEC), also referred to as enterohemorrhagic *E.coli* (EHEC) and verocytotoxic *E.coli* (VTEC), cause approximately 265,000 illnesses annually in the United States, with more than 3,600 associated hospitalizations and 30 deaths. A particularly virulent strain of STEC, O157:H7, accounts for about 75% of these illnesses. Non-O157 STEC are also responsible for illness in the U.S. and throughout the world. The most commonly identified non-O157 serotypes in the U.S. include O26, O45, O103, O111, O118, O121, and O145. The CDC recommends testing all stool cultures for Shiga toxins and The Joint Commission mandates all member labs must test all stool cultures for O157 STEC. Antibiotics should not be used to treat STEC infections as there is no evidence that antibiotics shorten the duration of illness and antibiotics may increase the risk of hemolytic uremic syndrome.

Enteroaggregative *E. coli* (EAEC)—Defined by their “stacked brick” aggregative adherence pattern when observed on cultured cells, this phenotypic description of the pathotype results in a heterogeneous and highly divergent group of *E.* *coli*. Transmission of EAEC is generally by the fecal-oral route via contaminated food and water. EAEC cause an inflammatory diarrheal illness characterized by watery and sometimes bloody stool, accompanied by low-grade fever, vomiting, and abdominal pain. EAEC infections may also be asymptomatic. Based upon various studies, EAEC are suggested to be one of the most common causes of diarrheal illness in the US across all age groups, a cause of persistent diarrhea in children and HIV-infected individuals, the second most common cause of travelers’ diarrhea, and has been identified as the cause of large outbreaks worldwide.

Enteropathogenic *E. coli* (EPEC)—Enteropathogenic *E. coli* do not produce enterotoxins or Shiga-like toxins. Rather, EPEC contain additional virulence factors including those encoded by the chromosomal locus of enterocyte effacement (LEE) pathogenicity island. EPEC has previously been associated with several deadly outbreaks at hospital nurseries in developed countries. Outbreaks appear to peak in the warmer months of summer and early fall. Illness caused by typical EPEC is associated with acute diarrhea whereas atypical EPEC cause a prolonged, non-bloody diarrhea, and vomiting with fever. When untreated in children, EPEC illness can lead to malnutrition and associated growth defects. Asymptomatic carriage of EPEC has also been documented with some studies reporting similar rates to symptomatic individuals.

Enterotoxigenic *E. coli* (ETEC)—The presence of heat-labile (*lt*) and/or heat-stable (*st*) enterotoxins defines Enterotoxigenic *E. coli* (ETEC). These toxins (which may be found together or separately in ETEC strains) bind to intestinal epithelial cells triggering loss of electrolytes resulting in watery diarrhea. ETEC are an important cause of diarrhea in developing countries especially among children, and are the most common bacterial cause of watery diarrhea in US travelers returning from abroad (commonly referred to as travelers’ diarrhea). ETEC infection remains significantly under-diagnosed and underreported due to the difficulty of identification and because infected adults may not seek treatment, as infections resolve in a few days with supportive care (rehydration). ETEC may also be carried asymptomatically.

Enteroinvasive *E. coli* (EIEC)*—*EIEC strains contain a plasmid encoding virulence factors (such as invasion plasmid antigen *ipaH*) that allow the bacteria to invade the colon and produce a watery diarrhea syndrome that is identical to that caused by Shigella. EIEC is rare in the US and EU and is also less common worldwide than ETEC and EPEC. Shigella and EIEC infections are generally treated in the same manner.

*Cryptosporidium*—this is a genus of protozoa capable of causing infections of the human stomach, intestine, and biliary ducts following ingestion of chlorine-tolerant oocysts that are shed in fecal material and can contaminate drinking water, recreational water, or food. *Cryptosporidium* are among the most common parasitic causes of diarrhea in developed nations. Illness is generally characterized by short-term gastroenteritis that resolves without treatment. However, severe illness is possible in immunocompromised individuals, particularly those with AIDS, where illness resolves slowly or not at all and can be fatal.

*Cyclospora cayetanensis*—this is a parasitic protozoa that causes gastroenteritis in humans, which are the only known hosts. Unsporulated oocysts are disseminated in feces. After a period of maturation (days to weeks), the oocysts become infectious and can cause illness if ingested through contaminated food or water. Infections are most common in tropical, subtropical, or warm temperate regions. In the US infections are associated with travelers’ diarrhea in persons returning from endemic areas. Additionally, outbreaks have been associated with consumption of contaminated food from other countries. There are an estimated 11,000 foodborne illnesses due to *C. cayetanensis* infections annually in the US but the true incidence may be underestimated due to the difficulty of diagnosing infection. Illness presents as non-bloody diarrhea that may be up to several months in duration.

*Entamoeba histolytica*—A pathogenic protozoa found worldwide with a particularly high prevalence in tropical and subtropical regions. *E. histolytica* cysts are generally ingested from materials contaminated with feces, such as food and water, but infection may also be transmitted sexually. Humans are the primary reservoir. Most infections from *E.* *histolytica* appear to be asymptomatic but some infections cause invasive amebiasis, which results in colitis or dysentery-like illness that can be severe and include amebic liver abscess.

*Giardia lamblia****—***Also referred to as *G. duodenalis* and *G. intestinalis*, these intestinal flagellate parasites are found worldwide. *Giardia* are the most common intestinal parasites isolated in the US and EU and are a leading cause of parasitosis worldwide. Populations with the highest risk of *G. lamblia* infection include children in day care centers, hikers, and the immunocompromised. The majority of *G. lamblia* infections are asymptomatic, but those who develop illness experience nausea, fever, and watery diarrhea. Infections are generally self-limiting; though symptoms are long-lasting, and some patients go on to develop chronic illness, which can lead to complications.

Adenovirus F 40/41*—*Adenoviruses are double-stranded DNA viruses of the *Adenoviridae* family that cause a variety of diseases including respiratory illness and gastrointestinal illness. They are resistant to chemical and physical damage and are thus persistent in the environment, facilitating transmission. Transmission is mostly through fecal-oral spread and outbreaks have been reported in hospitals and child care centers. While Adenovirus infections mostly occur in children, adults may be affected as well. Illness is generally mild but of a relatively long duration (5-12 days). Immunocompromised patients may suffer chronic, prolonged diarrheal illness and other complications. Virus may be shed in stool for weeks to months following acute illness; therefore identification of infected individuals may be important for patient isolation and control of disease spread.

Astrovirus—Astroviruses (RNA viruses of the family *Astroviridae*) are named for their characteristic star-like structure and are found in a variety of animals, including birds and mammals. The infection route is fecal-oral and at-risk populations include children, immunocompromised adults, caregivers of sick children, military troops, and those in nursing homes Symptoms are reported to be milder than other enteric viruses and include diarrhea, vomiting, abdominal pain, and fever lasting 72 hours.

Norovirus GI/GII—Noroviruses are highly contagious and cause on average 19 - 21 million cases of acute gastroenteritis each year. Illness due to Norovirus is estimated to cost two billion dollars annually in the U.S, ranking Norovirus in the top five pathogens for enteric illnesses. Infection with Norovirus, a single-stranded non-enveloped RNA virus, causes nausea, vomiting, diarrhea, and abdominal pain. Norovirus infections constitute a major disease burden leading to high rates of hospitalization and mortality in children and the elderly. Five distinct Norovirus genogroups have been described (G1 – GV), but human pathogens have only been described from genogroup I, genogroup II and genogroup IV. However, genogroup IV is a rare cause of disease in the United States.

Rotavirus A—Globally, Rotavirus, a double-stranded non-enveloped RNA virus, is the leading cause of severe diarrhea in infants and young children. Symptoms include fever, vomiting and watery diarrhea and may last upwards of 8 days after initial infection. Vaccination efforts have greatly reduced the incidence of Rotavirus infection in the U.S. since 2006, when as estimated 60,000 children were hospitalized each year. Antiviral drugs are ineffective against Rotavirus and the best treatment is management of dehydration.

Sapovirus (Genogroups I, II, IV, and V)—Sapovirus is a *Calciviridae* family member that is similar to Norovirus both genetically and in disease presentation. There are five genogroups (GI–GV); groups GI, GII, GIV, and GV are known to infect humans. Sapovirus causes disease mostly in children, though adults are susceptible as well, with outbreaks reported in long-term care facilities, prisons, cruise ships, and hospitals in the US. Like Norovirus, Sapovirus is spread via the fecal-oral route and infections are highest during winter months. Symptoms primarily include vomiting and diarrhea with nausea and fever lasting 5 to 10 days. In general, illness is self-limiting with treatment consisting of supportive care

**XII. Limitations**

1. A trained health care professional should interpret assay results together with the patient’s medical history, clinical signs and symptoms, and the results of other diagnostic tests.
2. For prescription use only.
3. FilmArray Gastrointestinal (GI) Panel performance has only been established on the FilmArray, FilmArray 2.0, and FilmArray Torch systems.
4. This test is a qualitative test and does not provide a quantitative value for the organism(s) in the sample.
5. The performance of this test has only been validated with human stool collected in Cary Blair transport medium, according to the media manufacturers’ instructions. It has not been validated for use with other stool transport media, raw stool, rectal swabs, endoscopy stool aspirates, or vomitus.
6. This product should not be used to test stool samples in fixative (e.g., formalin or polyvinyl alcohol; PVA).
7. The performance of this test has not been established for patients without signs and symptoms of gastrointestinal illness.
8. Virus, bacteria, and parasite nucleic acid may persist *in vivo* independently of organism viability. Additionally, some organisms may be carried asymptomatically. Detection of organism targets does not imply that the corresponding organisms are infectious or that they are the causative agents for clinical symptoms.
9. Results from this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient. Due to high rates of asymptomatic carriage of *Clostridium difficile*, especially in very young children and hospitalized patients, the detection of toxigenic *C. difficile* should be interpreted within the context of guidelines developed by the testing facility or other experts (e.g., guidelines/policy statements published by The American Academy of Pediatrics or the Society for Healthcare Epidemiology of America and the Infectious Disease Society of America).
10. The performance of this test has not been established for monitoring treatment of infection with any of the panel organisms.
11. Discrepancies between the FilmArray GI Panel and other microbial identification methods may be caused by the inability to reliably differentiate species based on standard phenotypic microbial identification methods. Examples include differentiation of *Yersinia enterocolitica* from other *Y. enterocolitica* group members such as *Y. kristensenii* or *Y. fredricksonii,* differentiation of *Entamoeba histolytica* from *E. dispar*, and differentiation of *Helicobacter* *pullorum* from *Campylobacter*. See Organism Interpretation section of this document for other specific examples.
12. There is a risk of false negative values due to the presence of sequence variants in the gene targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organisms for amplification.
13. The identification of several diarrheagenic *E. coli* pathotypes has historically relied upon phenotypic characteristics, such as adherence patterns or toxigenicity in certain tissue culture cell lines. The FilmArray GI Panel targets genetic determinants characteristic of most pathogenic strains of these organisms but may not detect all strains having phenotypic characteristics of a pathotype. In particular, the FilmArray GI Panel will only detect Enteroaggregative *E. coli* (EAEC) strains carrying the *aggR* and/or *aatA* genes on the pAA (aggregative adherence) plasmid; it will not detect all strains exhibiting an aggregative adherence pattern.
14. Target genes associated with the diarrheagenic *E. coli*/*Shigella* pathotypes are capable of horizontal transfer between strains, thus Detected results for multiple diarrheagenic *E. coli/Shigella* may be due to co-infection with multiple pathotypes or, less frequently, may be due to the presence of a single organism containing genes characteristic of multiple pathotypes. An example of the latter is the 2011 *E. coli* O104:H4 outbreak strain that contains determinants of both STEC and EAEC.
15. The FilmArray GI Panel detects the heat-labile toxin (LT) and heat-stable toxin variants (ST1a and ST1b) of Enterotoxigenic *E. coli* (ETEC), which are associated with human disease. The variant LT-II toxin (structurally similar to LT) and the STB/ST2 toxin (structurally dissimilar to ST1) are not targeted by the ETEC assays and have not been established as important in human disease.
16. The FilmArray GI Panel detects Enteropathogenic *E. coli* (EPEC) through targeting of the *eae* gene, which encodes the adhesin intimin. As some Shiga-like toxin-producing *E. coli* (STEC) also carry *eae* (in particular, strains identified as enterohemorrhagic *E. coli*; EHEC), the FilmArray GI Panel cannot distinguish between STEC containing *eae* and a co-infection of EPEC and STEC. Therefore, the EPEC result is not applicable (N/A) and not reported for specimens in which STEC has also been detected. In rare cases, STEC may be reported as EPEC when an STEC carrying *eae* (EHEC) is present in a specimen below the LoD of the STEC assay(s), or the strain carries an *stx* variant that is not detected well by the STEC assay(s) (e.g. *stx2* variant f).. Rare instances of other organisms carrying *eae* have been documented; e.g., *Aeromonas* spp., *Citrobacter* spp., *Escherichia albertii*, and *Shigella boydii*.
17. *Shigella dysenteriae* possess a shiga toxin gene (*stx*) that is identical to the *stx1* gene of STEC. The detection of both *Shigella*/Enteroinvasive *E. coli* (EIEC) and STEC *stx1/stx2* analytes in the same specimen may indicate the presence of *S. dysenteriae*. Rare instances of the detection of shiga-like toxin genes in other genera/species have been reported; e.g., *Aeromonas caviae*, *Acinetobacter haemolyticus*, *Shigella sonnei*, *Enterobacter cloacae*, *Citrobacter freundii*, and *Klebsiella pneumoniae*.
18. The *E. coli* O157 result is only reported in association with STEC *stx1/stx2*. While non-STEC O157 strains have been detected in human stool, their role in disease has not been established. Serotype O157 EPEC strains have been identified and will be detected by the FilmArray GI Panel (by the EPEC assay) due to their carriage of the *eae* gene.
19. The FilmArray GI Panel cannot distinguish between infections with a single toxigenic STEC O157 or rare coinfections of STEC (non-O157) with an *stx1/stx2*-negative *E. coli* O157.
20. This test only detects *Campylobacter jejuni*, *C. coli* and *C. upsaliensis* and does not differentiate between these three species of Campylobacter. Additional testing is required to differentiate between these species and to detect other Campylobacter species that may be present in stool specimens.
21. The detection of organism nucleic acid is dependent upon proper sample collection, handling, transportation, storage, and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false positive and false negative results caused by improperly collected, transported, or handled specimens. The RNA process control and the PCR 2 control will not indicate whether or not nucleic acid has been lost due to inadequate collection, transport or storage of specimens.
22. Due to the complex and highly variable nature of stool specimens, freezing may affect analyte integrity and subsequent test results for some specimens.
23. A negative FilmArray GI Panel result does not exclude the possibility of gastrointestinal infection. Negative test results may occur from sequence variants in the region targeted by the assay, the presence of inhibitors, technical error, sample mix-up, or an infection caused by an organism not detected by the panel. Test results may also be affected by concurrent antimicrobial therapy or levels of organism in the sample that are below the limit of detection for the test. Negative results should not be used as the sole basis for diagnosis, treatment, or other management decisions.
24. Organism and amplicon contamination may produce erroneous results for this test. Particular attention should be given to the Laboratory Precautions noted under the Warnings and Precautions section.
25. If four or more distinct organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.
26. The performance of the FilmArray GI Panel has not been established in individuals who received Rotavirus A vaccine. Recent oral administration of a Rotavirus A vaccine may cause positive results for Rotavirus A if the virus is passed in the stool.
27. The effect of interfering substances has only been evaluated for those listed in the labeling. Interference by substances other than those described in the Interference section below could lead to erroneous results.
28. Several organisms were shown to have the potential to cross-react with FilmArray GI Panel assays. These include *Entamoeba dispar* when present at high levels (*E. histolytica* assay); *Bifidobacterium* spp. and *Ruminococcus* spp. (*G. lamblia* assay); certain strains of *Citrobacter koseri*, *Citrobacter sedlakii*, *Hafnia alvei*, and *Cedeceae davisiae* containing variants of a flagellar assembly protein (ETEC 2 assay), *E. coli* containing a variant type III secretion protein (*Salmonella* assay), *Grimontia hollisae* which was formerly classified as a *Vibrio* sp. (*Vibrio* assay), *Yersinia frederiksenii* and *Yersinia kristensenii*, which are members of the *Y. enterocolitica* group (*Y. enterocolitica* assay). Please refer to the Organism Interpretation and Analytical Specificity sections of this document for additional information.
29. Cross-reactivity with organisms other than those listed above or in the Organism Interpretation or Analytical Specificity sections may lead to erroneous results.
30. *Campylobacter* inclusivity testing and *in silico* analyses demonstrated that the FilmArray GI Panel may have variable detection or reduced sensitivity for some organisms detected by the *Campylobacter* assays (Note: The *Campylobacter* assays only detect *C. jejuni*, *C. coli*, and *C. upsaliensis*). *Campylobacter upsaliensis* strain ATCC 43954 and *Campylobacter jejuni* subsp. *doylei* may not be detected and *in silico* analysis indicates primer mismatches that might lead to reduced assay sensitivity or lack of reactivity with 11/138 *C. coli* sequences currently in NCBI databases.
31. Empirical testing and *in silico* sequence analysis indicate that the *Vibrio* assay (*V. parahaemolyticus*/*V. vulnificus*/*V. cholerae*) may react with some less common *Vibrio* species (i.e., *V. alginolyticus*, *V. fluvialis*, and *V. mimicus*)but it is not expected to detect the rarer *Vibrio cincinnatiensis, Vibrio furnissii*, and *Vibrio metschnikovii* (Note: *Vibrio* spp. not associated with human disease were not evaluated).
32. *V. cholerae* isolates with highly divergent *toxR* genes will be non-reactive with the FilmArray GI Panel *V. cholera* assay. Additionally, very rare strains of pathogenic *V. cholerae* that do not carry that *toxR* gene will also not bedetected by the Vchol assay.
33. Rare isolates of *V. harveyi*, *V. mimicus*, and *V. vulnificus* that have acquired a homolog of the *toxR* gene have been reported and may show cross-reactivity with the Vchol assay.
34. Based on the available sequences, a few *Cryptosporidium* species, or certain variants of species, including *C. bovis*, *C. ryanae*, and *C. xiaoi*, may not be efficiently detected by the *Cryptosporidium* assays. These species arerarely detected in human samples.
35. There is a risk of false negative results due to the presence of strains with sequence variability or genetic rearrangements in the target regions of the assays. Refer to the inclusivity testing section of this document for additional information.
36. Unexpected results obtained from testing isolates from culture collections (e.g., during quality control testing) may occur due to mislabeling or miscategorization of the isolate, contamination of the stock, or genetic rearrangements (including loss of virulence plasmids) during repeated passaging.
37. Not all *Salmonella* serotypes were tested in validation studies; however, representatives of the 20 most prevalent serotypes recently circulating in the US (CDC National *Salmonella* Surveillance Annual Summary 2009) were evaluated. *In silico* sequence analysis supports detection of all subspecies and serotypes of *Salmonella*.
38. Cross-re*activity w*ith the *Salmonella* assay may occur with certain *E. coli* strains containing variants of the cryptic ETT2 type-III secretion system (see Inclusivity for *additional* information).
39. Positive and negative predictive v*alues are* highly dependent on prevalence. False negative results are more likely d*uring peak* activity when prevalence of *disease is* high. False positive results *are more* likely during periods when prevalence is moderate to low.
40. The performance of this test has not been evaluated for immunocompromised individuals.

**XIII. References**

FilmArray Gastrointestinal (GI) Panel Instruction Booklet, April 3, 2016.

Package Insert, FilmArray GI Control Panel M238, Maine Molecular Quality Controls, Inc, Saco, Maine, M238 07132016.003.

Biofire Torch Operator’s Manual. HTFA-PRT-0001-02. May 2016.