### FilmArray® Gastrointestinal Panel Testing

### Purpose

This procedure provides instructions for testing stool samples in Cary Blair transport media using the FilmArray Gastrointestinal Panel (GI) Kit.

### Background

The FilmArray GI is a multiplexed nucleic acid test intended for use with the FilmArray Instrument for the simultaneous qualitative 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 following bacteria (including several diarrheagenic E. coli/Shigella pathotypes), parasites, and viruses are identified using the FilmArray GI Panel:

\*\**Clostridium difficile (C. difficile)* toxin A/B will not be reported and must be ordered individually.

* *Campylobacter* (*C. jejuni/C. coli/C. upsaliensis*)
* *Clostridium difficile* (*C. difficile*) toxin A/B
* *Plesiomonas shigelloides*
* *Salmonella*
* *Vibrio* (*V. parahaemolyticus/V. vulnificus/ V. cholerae*), including specific identification of *Vibrio cholerae*
* *Yersinia enterocolitica*
* Enteroaggregative *Escherichia coli* (EAEC)
* Enteropathogenic *Escherichia coli* (EPEC)
* Enterotoxigenic *Escherichia coli* (ETEC) *lt/st*
* Shiga-like toxin-producing *Escherichia coli* (STEC) *stx1/stx2* (including specific identification of the *E. coli* O157 serogroup within STEC)
* *Shigella*/ Enteroinvasive *Escherichia coli* (EIEC)
* *Cryptosporidium*
* *Cyclospora cayetanensis*
* *Entamoeba histolytica*
* *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*)
* Adenovirus F 40/41
* Astrovirus
* Norovirus GI/GII
* Rotavirus A
* Sapovirus (Genogroups I, II, IV, and V)

### Principle of the Procedure

### The FilmArray GI pouch is a closed system disposable 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, and starts the run. All other operations are automated.

### 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). 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 of this booklet.

### The FilmArray Software controls the operation of the instrument, collects and analyzes data, and automatically generates a test report at the end of the run.

### Specimen

**Human stool collected in Cary Blair transport medium.**

* Stool specimens should be collected in Cary Blair transport media. Transfer raw stool into vial within 2 hours of collection. If a small amount of stool is received make a 3 parts Cary Blair media: 1 part stool diltion in a 15mL conical tube for testing.
* 200 µL of sample is required for testing.
* Specimens in Cary Blair should be processed and tested as soon as possible, though they may be stored at room temperature or under refrigeration for up to four days.

### Materials

|  |  |
| --- | --- |
| **Materials Provided** | **Materials Required But Not Provided** |
| Each kit contains sufficient reagents to test 30 or 6 specimens:* Individually packaged FilmArray GI 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
 | FilmArray System including:FilmArray Instrument and software FilmArray Pouch Loading Station compatible with the use of the FilmArray Injection VialsNote: Previous versions of Pouch Loading Station should not be used with the FilmArray Injection Vials. |

### Quality Control

**Process Controls**

Two process 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.

1. **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.

**Monitoring Test System Performance**

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). If required by local, state, or accrediting organization quality control requirements, users can monitor the system by trending Tm values for the control assays and maintaining records according to standard laboratory quality control practices. The PCR2 Control is used in all pouch types and can therefore be used to monitor the system when multiple pouch types (e.g., RP, GI and BCID) are used on the same FilmArray Instrument.

**External Controls**

External controls are to be performed for new lot before use and 30 day QC protocols. FilmArray GI Panel M238 contains two controls that provide a positive and negative result for every target of the FilmArray RP assay. Record results in appropriate QC log.

### Procedure

Refer to the FilmArray Gastrointestinal Panel Quick Guide for a more detailed and pictorial representation of these instructions.

Gloves and other Personal Protective Equipment (PPE) should be used when handling pouches and specimens. Only one FilmArray GI pouch should be loaded at a time. Once the pouch is loaded, it should be promptly transferred to the instrument to start the run. After the run is complete, the pouch should be discarded in a biohazard container.

**Prepare Pouch**

1. Thoroughly clean the work area and the FilmArray Pouch Loading Station with freshly prepared 10% bleach (or suitable disinfectant) followed by a water rinse.
2. Obtain the following required materials and place in the clean hood:
	* FilmArray GI Panel pouch
	* Sample Buffer ampoule
	* Hydration Injection Vial (blue cap)
	* Sample Injection Vial (red cap)
	* Transfer pipette
3. Place a blue capped Hydration Injection Vial in the blue well of the Pouch Loading Station.
4. Place a red capped Sample Injection Vial in the red well of the Pouch Loading Station.
5. Obtain patient sample and place into hood.
6. Remove the FilmArray GI pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective aluminum canister.

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

1. 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 base of the Pouch Loading Station.

**Hydrate Pouch**

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. 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.
2. 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 fails to hydrate, retrieve a new pouch and repeat from Step 2 of the Prepare Pouch Section.
3. Discard the Hydration Injection Vial in a suitable puncture proof container.

**Prepare Sample Mix**

1. Hold the Sample Buffer ampoule so that the tip is facing up.

**NOTE: Use care to avoid touching the tip during handling, as this may introduce contamination.**

1. Gently pinch the textured plastic tab on side of ampoule until the seal snaps.
2. 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.
3. Thoroughly mix the patient specimen.
4. Using the transfer pipette provided in the test kit, draw sample to the second line (approximately 0.2 mL). Add sample to the red Sample Injection Vial.

**NOTE: DO NOT use the transfer pipette to mix the sample once it is loaded into the Sample Injection Vial.**

1. Tightly close the lid of the Sample Injection Vial and mix by gently inverting at least 3 times.
2. Return the Sample Injection Vial to the Pouch Loading Station.

**Load Sample Mix**

1. Slowly unscrew 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.**

1. 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.
2. 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.
3. Discard the Sample Injection Vial in a suitable biohazard and puncture proof container. Do not re-cap the vial.
4. Record the Sample ID in the provided area on the pouch label (or affix a barcoded Sample ID) and remove the pouch from the Pouch Loading Station.

**Run Pouch**

The FilmArray Instrument Control Software includes a step-by-step on-screen tutor that shows each step of the test.

1. Ensure that the computer and FilmArray Instrument have been turned on. Launch the FilmArray Instrument Control Software by double clicking on the desktop icon.
2. Open the instrument lid (if not already open).
3. Insert the loaded FilmArray pouch into the instrument.

Position the pouch so that the array is on the right and the film is inserted first. The red and blue labels on the FilmArray pouch should align with the red and blue arrows on the FilmArray Instrument. The pouch will click into place. If inserted correctly, the barcode is visible and the label is readable on the top of the pouch. The instrument and software must detect that the pouch has been inserted correctly before continuing to the next step.

**NOTE: If the pouch does not slide into the instrument easily, gently push the lid of the instrument back to be sure that it is completely open.**

1. Scan the barcode on the FilmArray pouch using the barcode scanner.

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. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.



**NOTE: The barcode cannot be scanned prior to placing the pouch in the instrument. A “Cannot scan now” message will be displayed.**

**A “Cannot scan now” message will be displayed.**

1. Enter the Sample ID.

The Sample ID can be entered manually or scanned in by using the barcode scanner when a barcoded Sample ID is used.

1. If necessary, select a protocol from the Protocol drop down list.
2. Enter a user name and password in the Name and Password fields.
3. Close the FilmArray Instrument lid.
4. Click Start Run.

Once the run has started, the screen displays a list of the steps being performed by the instrument and the number of minutes remaining in the run.

**NOTE: The bead-beater apparatus can be heard as a high-pitched noise (whine) during the first minute of operation.**

1. When the run is finished, results are automatically displayed in the report section of the screen. The report is automatically saved into the database.
2. Select **Print** to print the report, or **Save** to save the report as a PDF file.
3. Follow the on-screen instructions to open the instrument and remove the pouch.
4. Immediately discard the pouch in a biohazard container.

### Interpretation

The FilmArray Software automatically analyzes and interprets the assay results and displays the final results in a test report (see the FilmArray Gastrointestinal Panel Quick Guide to view an example of a test report). The analyses performed by the FilmArray Software and details of the test report are described below.

**Assay Interpretation**

When 2nd stage PCR is complete, the FilmArray Instrument performs a high resolution DNA melting analysis on the PCR products and measures the fluorescence signal generated in each well (for more information see FilmArray Operator’s Manual). The FilmArray Software then performs several analyses and assigns a final assay result.

Analysis of melting curves.The FilmArray Software evaluates the DNA melting 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 is positive and the melt peak falls inside the assay-specific Tm range, the curve is called positive. If the software determines that the melt is negative or is not in the appropriate Tm range, the curve is called negative.

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 curves must be similar (within 1°C). Assays that do not meet these criteria are called negative.

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). Interpretation rules for these assays are described in the Gastrointestinal Panel Instruction Booklet.

**NOTE: If four or more distinct organisms are detected in a specimen, retesting is recommended to confirm polymicrobial results.**

**FilmArray 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 (see the FilmArray Gastrointestinal Panel Quick Guide to view an example of a test report). 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 than 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 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.

**Control 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 ANDBoth pouch controls were successful. | None | Report the results provided on the test report. |
| Failed | The run was successfully completed BUTAt 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. |

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### Result Reporting

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. Reporting of Results and Required Actions

| **Result** | **Explanation** | **Action** |
| --- | --- | --- |
| Detected | The run was successfully completedANDThe pouch controls were successful (Passed)ANDThe 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 results. |
| Not Detected | The run was successfully completedANDThe pouch controls were successful (Passed)ANDThe assay(s) associated with the interpretation were negative (did not meet the requirements for a positive assay described in Detected). | None. Report results. |
| N/A(applies to *E. coli* O157 and EPEC only) | The run was successfully completedANDThe pouch controls were successful (Passed)ANDFor *E. coli* O157: Shiga-like toxin-producing *E. coli* was Not Detected.For EPEC: Shiga-like toxin-producing *E. coli* was Detected. | None. Report results. |
| Invalid | The run did not complete successfully (Aborted, Incomplete, Instrument Communication Error, Instrument Error, or Software Error)ORThe pouch controls were not successful (Failed). | See Table 1, *Interpretation of Controls Field on the FilmArray GI Test Report*, for instruction. |

Positive reporting:

* + - 1. Choose keypad Key P
			2. Choose positive Organism(s)
			3. Chose keypad Key C
			4. Issue Final Report





\*\*\*Note: The above rule will appear if positive is reported. Salmonella, Shigella, EC0157,STEC or Vibrio must be called immediately to caregiver and Infection Prevention. The original sample must be sent to IDPH for confirmation. 2nd and 3rd shift leave sample on bench with result for 1st shift. Note “Called to” information in the callbox.

\*\*\*Note: If *Shigella/*Enteroinvasive *E. coli*(EIEC) Detected. Sub to GN, MAC, BAP and attempt to isolate/perform ID/MIC on *Shigella* before sending to IDPH.

Equivocal reporting when verified by repeat testing:

1. Choose keypad Key E
2. Choose Equivocal Organism(s)
3. Chose keypad Key C
4. Issue Final Report

Negative reporting:

1. Choose keypad Key N
2. Issue Final Report

Internal QC failure

1. Repeat test using same sample on different module.
2. If 2nd internal QC failure occurs. Cancel test using the following cancel reason “Multiple internal QC failures. Recollection required. Called to information”

\*\*\* NOTE: BioFire Diagnostics, LLC, is aware of an increased rate of false positive results in assays to detect *Campylobacter* and *Cryptosporidium* in the BioFire GI Panel due to a low-rate manufacturing error that causes non-specific amplification of PCR products. It is important to note that only the *Campylobacter* and *Cryptosporidium* assays are affected by this error, due to their susceptibility to non-specific amplification, and that no other assays in the BioFire GI Panel are at risk for false positive results for this specific error.

As a result, BioFire recommends retesting positive results for *Campylobacter* and *Cryptosporidium* with a second BioFire GI Panel test before reporting in order to decrease the likelihood of false positive test results. Using this testing scheme true positive *Campylobacter* and *Cryptosporidium* will be confirmed with the second positive result. If a negative result is observed in either test, the sample is assumed to be a true negative, and the positive result is assumed to be caused by the manufacturing error.

### References/Related Documents

FilmArray Gastrointestinal Panel (GI) Instruction Booklet (RFIT-PRT-0143-01), BioFire Diagnostics, LLC.