### FilmArray® Meningitis/Encephalitis Panel Testing

### Purpose

This procedure provides instructions for testing cerebrospinal fluid (CSF) using the FilmArray Meningitis/Encephalitis Panel (ME) Kit.

### Background

The FilmArray Meningitis/Encephalitis (ME) Panel is a qualitative multiplexed nucleic acid-based in vitro diagnostic test intended for use with FilmArray systems. The FilmArray ME Panel is capable of simultaneous detection and identification of multiple bacterial, viral, and yeast nucleic acids directly from CSF specimens obtained via lumbar puncture from individuals with signs and/or symptoms of meningitis and/or encephalitis. The following organisms are identified using the FilmArray ME Panel:

**Bacteria:**

* *Escherichia coli* K1
* *Haemophilus influenzae*
* *Listeria monocytogenes*
* *Neisseria meningitidis* (encapsulated)
* *Streptococcus agalactiae*
* *Streptococcus pneumoniae*

**Viruses:**

* Cytomegalovirus
* Enterovirus
* Herpes simplex virus 1
* Herpes simplex virus 2
* Human herpesvirus 6
* Human parechovirus
* Varicella zoster virus

**Yeast:**

* *Cryptococcus neoformans/gattii*

### Principle of the Procedure

The FilmArray ME pouch is a closed system disposable that houses all the chemistry required to isolate, amplify and detect nucleic acid from multiple meningitis and encephalitis pathogens within a single CSF specimen obtained from a lumbar puncture. The rigid plastic component (fitment) of the FilmArray ME 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 ME Panel loads the sample into the FilmArray ME 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 agitation (bead beating) 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 minute of operation.
2. Reverse Transcription and 1st Stage Multiplex PCR - Some pathogens identified by the FilmArray ME pouch are RNA viruses, and 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 Defense, 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 of this booklet.

### Specimen

### CSF Specimen Collection

### CSF specimens should be collected via lumbar puncture, and should not be centrifuged.

### Minimum Sample Volume - 200 μL of CSF specimen is required for testing.

### Transport and Storage - Specimens should be processed and tested with the FilmArray ME Panel as soon as possible, though they may be stored for up to one day at room temperature, or under refrigeration for up to seven days.

### Materials

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| **Materials Provided** | **Materials Required But Not Provided** |
| Each kit contains sufficient reagents to test 30 or 6 specimens:   * Individually packaged FilmArray ME 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 Vials  Note: 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 ME 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. When 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.78,79 Refer to FilmArray Operator’s Manual for instructions on obtaining control assay Tm values. The PCR2 Control is used in all FilmArray pouch types (e.g., RP, GI, ME, and BCID) and can therefore be used to monitor the system when multiple pouch types are used on the same FilmArray system or instrument.

### Good laboratory practice recommends running external positive and negative controls regularly. Molecular grade water, or artificial CSF, can be used as an external negative control. Previously characterized positive CSF samples or negative samples spiked with well characterized organisms can be used as external positive controls. External controls should be used in accordance with the appropriate accrediting organization requirements, as applicable.

### Procedure

Refer to the FilmArray Meningitis/Encephalitis Panel Quick Guide, the FilmArray Training Video, or the FilmArray Operator’s Manual for more detail and pictorial representations of these instructions.

Gloves and other Personal Protective Equipment (PPE) should be used when handling pouches and samples. Only one FilmArray ME pouch should be prepared at a time. Once sample is added to the pouch, 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. Remove the 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 FilmArray Pouch Loading Station so that the red and blue labels on the pouch align with the red and blue arrows on the FilmArray Pouch Loading Station.
2. Place a blue-capped Hydration Injection Vial in the blue well of the FilmArray Pouch Loading Station.
3. Place a red-capped Sample Injection Vial in the red well of the FilmArray Pouch Loading Station.

Hydrate Pouch

1. Twist and lift the Hydration Injection Vial, leaving blue cap in the well of the FilmArray Pouch Loading Station.
2. Insert the cannula tip into the port in the pouch located directly below the blue arrow of the FilmArray 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 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), repeat Step 2 to verify that the seal of the port was broken or retrieve a new pouch and repeat from Step 2 of the Prepare Pouch section.

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.**

2. Gently pinch the textured plastic tab on the side of the ampoule until the seal snaps.

3. Invert the ampoule over the red-capped Sample Injection Vial and re-position thumb and forefinger to grip the bottom of the ampoule. Dispense Sample Buffer using a slow, forceful squeeze, followed by a second squeeze. Squeezing the ampoule additional times will generate excessive bubbles, which should be avoided.

4. Thoroughly mix the patient sample.

5. Using the Transfer Pipette provided in the test kit, draw cerebrospinal fluid (CSF) sample to the second line (approximately 0.2 mL) of the Transfer Pipette. Add the sample to the Sample Buffer in the Sample Injection Vial. Discard the Transfer Pipette in a biohazard waste container and tightly close the lid of the Sample Injection Vial.

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

6. Remove the Sample Injection Vial from the FilmArray Pouch Loading station and gently invert the vial at least 3 times to mix.

7. Return the Sample Injection Vial to the FilmArray Pouch Loading Station.

Load Sample Mix

1. Slowly twist the Sample Injection Vial so it loosens from its red cap and pause for 3-5 seconds. Lift the Sample Injection Vial, leaving the red cap in the well of the FilmArray Pouch Loading Station.

2. Insert the cannula tip into the port in the pouch fitment located directly below the red arrow of the FilmArray 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 the Prepare Pouch section. Discard the Sample Injection Vial and the Hydration Injection Vial in an appropriate biohazard sharps container.

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

5. Record the Sample ID in the provided area on the pouch label (or affix a barcoded Sample ID) and remove the pouch from the FilmArray Pouch Loading Station.

Run Pouch

1. Ensure that the FilmArray device is powered on and ready for use.

2. Follow on-screen instructions and procedures described in the Operator’s Manual to place the pouch in an instrument, enter pouch, sample and operator information, and start the run.

Pouch identification (Lot Number and Serial Number), Pouch Type and Protocol are preprogrammed in the rectangular barcode located on the FilmArray pouch. The information 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 into the appropriate fields. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.

3. 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.

4. If necessary, select the appropriate protocol for your sample type from the Protocol drop down list.

5. Enter a user name and password in the Name and Password fields.

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

6. Start run.

7. When the run is finished, follow the on-screen instructions to remove the pouch and immediately discard the pouch in a biohazard container.

8. The run file is automatically saved in the FilmArray database and the results report can be viewed, printed, and/or saved as a PDF file.

### Interpretation

The FilmArray software automatically analyzes and interprets assay results and displays the final results in a test report (see the FilmArray Meningitis/Encephalitis 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. The steps in the analyses are described below.

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.

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.

Organism Interpretation**.** The reported FilmArray ME Panel organism results (Detected or Not Detected) are based on analysis and interpretation of a single assay (most organisms) or a combination of two assays (*Haemophilus influenzae*, Herpes simplex virus 2 and Varicella zoster virus). For results that rely on two assays, a Detected result is reported when either one or both assays are positive and a Not Detected result is reported only when both assays are negative.

**NOTE**: Non-K1 *E. coli* serotypes may be present in a specimen and will not be detected by the FilmArray ME Panel.

**NOTE**: Non-encapsulated strains of *Neisseria meningitidis* are not detected by the FilmArray ME Panel.

**NOTE:** The FilmArray ME Panel does not distinguish between latent and active CMV and HHV-6 infections. Detection of these viruses may indicate primary infection, secondary reactivation, or the presence of latent virus. Results should always be interpreted in conjunction with other clinical, laboratory, and epidemiological information.

FilmArray ME Panel Test Report

The FilmArray ME Panel 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 Meningitis/Encephalitis 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 organism assays 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, 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.

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 ME Panel 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. | Accept the valid results of the repeat testing. If the error persists, contact Technical Support for further instruction. |

### Result Reporting

The Result Summary section provides a complete list of the test results. Possible results for each organism include Detected, Not Detected, 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 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. | Report results.  NOTE: If Detected results are reported for 2 or more organisms in a specimen, a retest of the specimen is recommended to confirm the polymicrobial result. |
| 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). | Report results. |
| 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 |

### References/Related Documents

FilmArray Meningitis/Encephalitis Panel (ME) CE-IVD Instruction Booklet (RFIT-PRT-0005), BioFire Diagnostics, LLC.