

***Department of Laboratory***

***Policy / Procedure***

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| **Title: MIC 4918.01—FilmArray Meningitis/Encephalitis Panel** | | **Original Date:**  **12/05/2017** | **Page:**  1 of 8 | |
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| **Keywords: FilmArray, CSFID, ME PCR,** | |  | | |
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**PURPOSE:** This procedure provides guidance for testing cerebrospinal fluid (CSF) using the FilmArray PCR Meningitis/Encephalitis Panel (ME) Kit, a qualitative, multiplexed nucleic acid based *in vitro* diagnostic test for use with the FilmArray instrument.

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 from individuals with signs and/or symptoms of meningitis and/or encephalitis.

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.

**POLICY:** Orders must be placed in the computer system to run the FilmArray ME Panel**, but it will be our policy to save at least 0.5 mL of unspun CSF in a sterile vial with number and patient identifiers on it in the Microbiology under the counter refrigerator for 7 days in case they want to go back and order the panel.** There will be a rack in the refrigerator in Micro. labeled for this purpose.

**The following organisms are identified using this FilmArray 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/gatti*

**(SPECIAL OR NURSING) CONSIDERATIONS:** CSF specimens should be collected as sterile as possible, not allowing for contamination of specimen in any way. When lumbar puncture is performed, CSF tubes should be filled and lids screwed on tightly as each tube is filled to avoid any respiratory flora contaminant from collector or assistant. Physician must wear gown, mask and sterile gloves when performing lumbar puncture and any one assisting should be similarly covered.

**EQUIPMENT:** FilmArray System including instrument and software, pouch loading station (designated for just ME Panel and washed before and after use with Cavacide or bleach), individually packaged FilmArray ME pouch, Sample Buffer ampule(1.0 mL) , Single-use pre-filled (1.5 mL) Hydration Injection Vial (blue), Single-use Sample Injection Vials (red), individually packaged transfer pipette.

**SPECIMEN: Cerebrospinal Fluid collected by lumbar puncture**. **Do not centrifuge**. Minimum Sample Volume = 200µL of CSF is required for testing. (**The FilmArray ME Panel is not intended for testing of specimens collected from indwelling CNS medical devices.)**

**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 up to 7 days under refrigeration.

**QUALITY CONTROL:**

Process Controls: Two process controls are included in each pouch:

Two process controls are included in each pouch: Internal Quality Control

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.

External Controls:

### 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.

Per IQCP, all negative and all positive quality control will be run with each new kit and every 6 weeks. Using Maine Molecular M262, **Introl ME** **Control Panel** which is shipped on dry ice. (23 Mill Brook Rd. Saco, ME 04072, phone: 207-885-1072, [www.mmqci.com](http://www.mmqci.com) ).**Store at -20°C.**

**PROCEDURE:**

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| **Step 1 Preanalytical** | 1. Don gloves and PPE. 2. Thoroughly clean the work area( biosafety cabinet) and the ME FilmArray Pouch Loading Station with freshly prepared 10% bleach, Cavicide wipe (or suitable disinfectant) followed by a water rinse. Change gloves. 3. (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.)   To remove canister and diluents from box, wear gloves to handle.  Place into Biosafety cabinet and **change gloves** before handling specimen and pouch. |
| **Step 2** | Remove the pouch from its vacuum-sealed package by cutting or tearing 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.** |
| **Step 3** | 1. Place the FilmArray pouch in the ME Loading Station. 2. 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. |
| **Step 4** | 1. Place the hydration fluid in the rack by the blue mark. 2. Place a blue-capped Hydration Injection Vial in the blue well of the FilmArray Pouch Loading Station. Slightly turn to free Vial from cap. Insert the cannula tip into the post in the pouch located directly below the blue arrow of the FilmArray Pouch loading Station. Push down forcefully in a firm, 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. Check that all wells have been hydrated evenly. |
| **Step 5** | 1. Place the Sample Injection Vial over the red mark on the loading rack. 2. Place a red-capped Sample Injection Vial in the red well of the FilmArray Pouch Loading Station.   **Place sample buffer on top of rack. Add the sample to the Sample Buffer in the Sample Injection Vial.**   1. Place Sample Buffer ampoule so that tip is facing up. Gently pinch the textured plastic on the side of the ampule until the seal snaps. 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 additional times may generate excessive bubbles, which should be avoided.) |
| **Step 6**  **Analytical** | **Add 0.2 mL of specimen into Sample Vial using pipet provided, to second line.**  Thoroughly mix the uncentrifuged patient sample. 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.  Discard Transfer Pipette in Biohazard Container and tightly close vial lid of Sample Injection Vial. (Do not use transfer pipette to mix the sample once it is loaded into the Sample Injection Vial.). |
| **Step 7** | **Mix specimen.**  Remove the Sample Injection Vial from the Pouch Loading Station and gently invert the vial 3-4 times to mix. |
| **Step 8** | **Return the Sample Injection Vial to the FilmArray Pouch Loading Station.**  **Slowly twist the Sample Injection Vial so that it loosens from its red cap and pause for 3-5 seconds.**  **Inject the sample/buffer into the hole on left side of the packet in loading rack.**  Lift the Sample Injection Vial from its red cap and insert cannula tip into the port in the pouch below the red arrow on the Pouch Loading Station. Push down forcefully in a firm, 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 |
| **Step 9** | **Flip barcode down to check fluid has entered the reagent well next to the sample loading port.**  **Place a patient barcode label on the flip-side of the test barcode.** |
| **Step 10** | Remove Hydration Buffer and Sample Injection Vial to individual caps and discard in biohazard container. |
| **Step 11** | **Change gloves.**  Remove the pouch from Loading Station. Take it to the instrument. |
| **Step 12** | **Load pouch in instrument.**  Remove gloves.  **Wand the barcode on the pouch.**  This will identify the Lot number, Serial number of instrument you are using, it will identify the test you are running. (Can be manually entered, if necessary.) |
| **Step 13** | **Wand the patient barcode.**  Type in the patient name after the barcode.  **Enter your user name and password in the appropriate fields and hit “Start Run”.** |
| **Step 14** | The run will take approximately 1 hour to run. When run is finished, follow the on-screen instructions to remove the pouch and immediately discard it into a biohazard container. |
| **Step 15** | The run file is automatically saved in the FilmArray database and the results report prints and is saved as a PDF file. |
| **Step 16**  **Post-Analytical** | After approximately 65 minutes, results should print.  **If two or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.** |
| **Step 17** | Go to the computer system and enter the results as we do for the BCID.  All results will get an “N” for “Not Detected” or a “D” for “Detected”. |
| **Step 18** | Call the results to the Emergency Ordering Physician or the Charge Nurse on the inpatient floor. |
| **Step 19** | Document using the “CT” Canned Text under Specimen Comments. |

**Post-Analytical:**

INTERPRETATION/RESULTS: The FilmArray software automatically analyzes and interprets assay results and displays the final results in a 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 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, 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.

**QUALITY ASSURANCE:**

**METHOD LIMITATIONS: Table 2. Reporting of Results and Required Actions**

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| **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 |

**PROCEDURE NOTES:** 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 infect**ions. 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.

**Note:** False negative results may occur when the concentration of organism(s) in the specimen is below the device limit of detection.

**Note:** If two or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.

**Note:** Only encapsulated forms of N.meningitidis and E.coli strains with the K1 capsular antigen will be detected.

**METHOD LIMITATIONS:**

1. Some organisms detected by the FilmArray ME Panel, such as *streptococcus pneumoniae* and *H. influenza* can be shed from respitatory tract of healthy individuals. HSV-1 may also be shed from individuals with active or recurrent cold sores. Particular attention should be given to the PPE used during collection. Caution should be exercised during specimen collection and testing to prevent contamination leading to false positive results.
2. HHV-6 or CMV can exist in latent form that is reactivated during infection due to other pathogens, including agents not detected by FilmArray ME panel that may cause meningitis/encephalitis (e.g., M*ycobacterium tuberculosis* or HIV). When detected by the FilmArray ME, HHV-6 or CMV should be considered as the likely cause of meningitis/encephalitis only in appropriate clinical setting and following expert consultation.
3. Viral shedding into CSF ofter occurs in cases of zoster (shingles; caused by reactivation of VZV). VZV may not be the cause of CNS disease in these cases.
4. Once pouch packaging has been opened, the pouch whould be loaded within 30 minutes.
5. Once pouch is loaded with sample, test run should be started as soon as possible (within 60 minutes),
6. If any liquid is seen on outside of pouch after testing, discard immediately an dcall unmber on the instrument for ful contamination instructions. Do not perform additional testing on that module until the area has been decontaminated.

**REFERENCE:**

May 02, 2016. RFIT-PRT-0276. FilmArray® Meningitis/Enchephalitis Panel (ME) CE-IVD Instruction Booklet. BioFire Diagnostics, LLC, 390 Wakara Way, Salt Lake City, UT 84108, USA.