Adventist HealthCare

Laboratories at Adventist Healthcare White Oak Medical Center, Shady Grove Medical Center, Fort Washington Medical Center and Germantown Emergency Center

Date: 2/11/22

LABORATORY ALERT

Subject:

The Laboratory is informing you of the following change:

Effective date:	February 15, 2022
SOP	BioFire® FilmArray® Meningitis/Encephalitis (ME) Panel (AHC.M1022)
Test Name:	Meningitis/Encephalitis (ME) Panel
Test Code:	BFME
Specimen Requirements:	CSF that **has not** been spun. If the tube used for Chemistry testing (glucose and protein) and has been spun down – we cannot use this tube to run on the BioFire because it could cause false results.
Additional information	An MTS has been assigned to SGMC and WOMC technical and non-technical staff to make sure that both lab sections are aware that **spun CSF cannot be used as an "add on" for this test.** If you have any questions please ask your lead tech or supervisor.
Contacts:	Stephanie Codina, Laboratory Manager 240 6015 Hollie Genser, SGMC Lab Supervisor 240-826-6432 Zanetta Morrow, WOMC Lab Supervisor 240-637-5681 Derrick Wallace, SGMC Field Ops Supervisor 240-826- 6642

Technical SOP

Title	**BioFire® FilmArray® Meningitis/Encephalitis (ME) Panel**
Prepared by	Ron Master Date: 5/20/2021
Owner	Ron Master Date: 5/20/2021

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

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1. TEST INFORMATION

Assay	BioFire® FilmArray® Meningitis/Encephalitis (ME) Panel
Method	Nested Multiplexed Polymerase Chain Reaction Assay
Instrument	BioFire FilmArray® Torch
Synonyms	BioFire® ME Panel, ME Panel
Department	Microbiology

Order Code	Test Name
BFME	Meningitis/Encephalitis (ME) Panel

2. ANALYTICAL PRINCIPLE

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.

The following organisms are identified using the BioFire ME Panel:

Bacteria	Viruses	Yeast
• *Escherichia coli* K1 • *Haemophilus influenzae* • *Listeria monocytogenes* • *Neisseria meningitidis* (encapsulated) • *Streptococcus agalactiae* • *Streptococcus pneumoniae*	• Cytomegalovirus (CMV) • Enterovirus (EV) • Herpes simplex virus 1 (HSV1) • Herpes simplex virus 2 (HSV2) • Human herpesvirus 6 (HHV6) • Human parechovirus (HPeV) • Varicella zoster virus (VZV)	• *Cryptococcus neoformans/gattii*

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	Not applicable
Specimen Collection and/or Timing	Lumbar puncture
Special Collection Procedures	**Specimen should not be centrifuged after collection**
Other	Not applicable

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Cerebrospinal Fluid (CSF) None
Collection Container	Sterile transport container
Volume - Optimum **- Minimum**	1 mL 0.2 mL
Transport Container & Temperature	Sterile transport container

Criteria		
Stability & Storage Requirements	Room Temperature	24 hours (15-25°C)
	Refrigerated	7 days (2-8°C)
	Frozen	Not acceptable
Timing Considerations	Not applicable.	
Unacceptable Specimens & Actions to Take	Reject all specimens below using the appropriate LIS message. • CSF collected from CSF shunts • Specimens in transport medium • Specimens past stability requirement • Centrifuged specimens • Specimens other than CSF	
Compromising Physical Characteristics	Leaking specimen.	
Other Considerations	Bleach can damage organisms/nucleic acids within the specimen, potentially causing false negative results. Contact between bleach and specimens during collection, disinfection, and testing procedures should be avoided.	

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation.

4. REAGENTS

The package insert for a new lot of kits or reagents must be reviewed for any changes before the kit is used.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
BioFire® FilmArray® Meningitis/Encephalitis (ME) Panel Test Kit	BioFire® RFIT-ASY-0118

4.2 Reagent Preparation and Storage

Assay Kit	
Kit Contents	BioFire® FilmArray® Meningitis/Encephalitis (ME) Panel Test Kit. Each kit contains sufficient reagents to test 30 samples • Individually packaged BioFire® FilmArray® Meningitis / Encephalitis (ME) Panel 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)
Storage	Store at room temperature 15 - 25°C. Do NOT refrigerate.
Stability	Until manufacturer's expiration date
Special Instructions	• All kit components should be stored and used together. • Do not use components from one kit with those of another kit. • Discard any extra components from the kit after all pouches have been used. • Always check the expiration date and do not use reagents beyond the expiration date printed on the pouch or kit.

5. CALIBRATORS/STANDARDS

Not applicable

6. QUALITY CONTROL

6.1 Controls Used

Quality Control	Supplier & Catalog Number?
External Controls MMQCI INTROL ME Control Panel (External Positive and Negative Controls)	Maine Molecular catalogue # M262
RNA Process Control	Cartridge component
PCR2 Control	Cartridge component

6.2 Control Preparation and Storage

Control	MMQCI FilmArray ME Control Panel (External Positive and Negative Controls)
Storage	-15°C or colder
Stability	Unopened: Until manufacturer's expiration Opened: One single use
Preparation	Control is supplied ready for use. No additional preparation required.

6.3 Number and Frequency

QC Frequency	
1	Internal Controls: RNA Process Control and PCR2 Control are run with each test
2	External Controls: • If no IQCP has been implemented, external QC will be performed with each new lot/shipment and each day of patient testing. • If an IQCP has been implemented, external QC will be performed with each new lot/shipment and at least every 31 thereafter (see IQCP QC Plan for details).

6.4 Tolerance Limits and Criteria for Acceptable QC

A. Tolerance Limits

Tolerance Limits		
Control Type		**Expected Result**
Internal Controls	RNA Process Control	**Positive –** 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 BioFire ME pouch were successful.
	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 PCR2 was successful.
	Melting Temperature (Tm)	RNA Process Control: 80.2-84.2°C PCR2 Control: 74.1-78.1°C

Tolerance Limits				
External Controls	MMQCI FilmArray ME Control Panel (External Positive and Negative Controls)	**Assay/Target**	**Positive M263**	**Negative M264**
		Bacteria		
		Escherichia coli K1	Detected	Not Detected
		Haemophilus influenzae	Detected	Not Detected
		Listeria monocytogenes	Detected	Not Detected
		Neisseria meningitidis	Detected	Not Detected
		Streptococcus agalactiae	Detected	Not Detected
		Streptococcus pneumoniae	Detected	Not Detected
		Viruses		
		Cytomegalovirus	Detected	Not Detected
		Enterovirus	Detected	Not Detected
		Herpes simplex virus 1	Detected	Not Detected
		Herpes simplex virus 2	Detected	Not Detected
		Human herpesvirus 6	Detected	Not Detected
		Human parechovirus	Detected	Not Detected
		Varicella zoster virus	Detected	Not Detected
		Yeast		
		Cryptococcus neoformans/gattii	Detected	Not Detected

B. Criteria for Acceptable QC
- All controls must yield acceptable results.
- Controls and patient data must be reviewed for acceptability and for atypical or unexpected results or trends prior to reporting patient results.
- DO NOT release results from runs with unacceptable controls or with unusual patterns, trends, or distribution in patient values.

C. Corrective Action
- Report problems to supervisor or designee.
- All rejected runs must be effectively addressed and include the following documentation:
    - Control(s) that failed (e.g., positive control with negative result) and/or atypical or unexpected patient results
    - Actions taken
    - Statement of what was done with the patient samples from the affected run/batch,
    - Date and initials of the person recording the information.
- Patient samples in failed analytical runs must be reanalyzed.

NOTE: The laboratory director or designee may override rejection of partial or complete runs. Justification for the override must be documented in detail.

6.5 Documentation

- Record all Quality Control results (failed and successful) manually or electronically.

- QC results and corrective action forms for this assay are documented on the respective QC forms.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

- BioFire FilmArray® Torch

7.2 Equipment

- FilmArray Pouch Loading Station
- Printer
- Biological Safety Cabinet (BSC)
- Refrigerator, 2-8°C
- Freezer, -15°C or lower

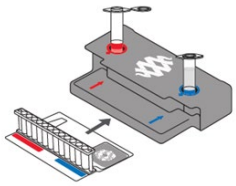
7.3 Supplies

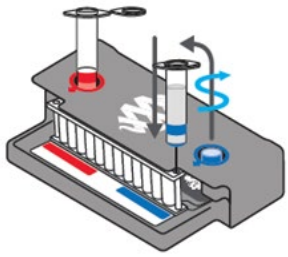
- 10% bleach solution or a similar disinfectant

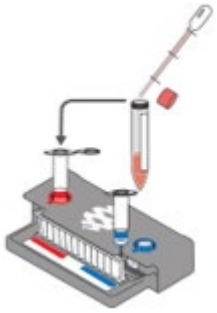
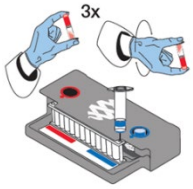
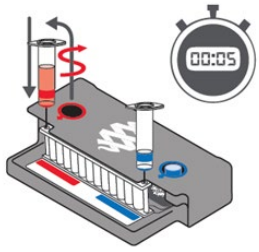
8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

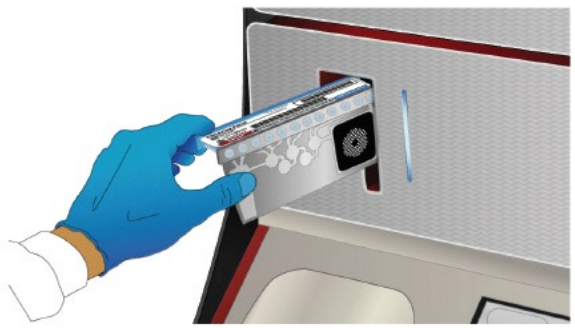
8.1	Specimen / Reagent Preparation
General Precautions	
Preventing organism contamination • Samples must be processed in a biological safety cabinet. • A biological safety cabinet used for CSF culture or other testing should not be used for ME panel sample preparation or pouch loading. • Prior to processing samples, thoroughly clean both the work area and the FilmArray Pouch Loading Station using 10% bleach. To avoid residue buildup and potential damage to the specimen or interference from disinfectants, wipe disinfected surfaces with water. • Wear a surgical mask throughout all steps in the procedure. • Samples and pouches must be handled one at a time. • Once sample is added to the pouch, promptly transfer the pouch to the instrument to start the run. • Use clean gloves to remove materials from bulk packaging bags and reseal bulk packaging bags when not in use. • Change gloves and disinfect the work area between each sample.	

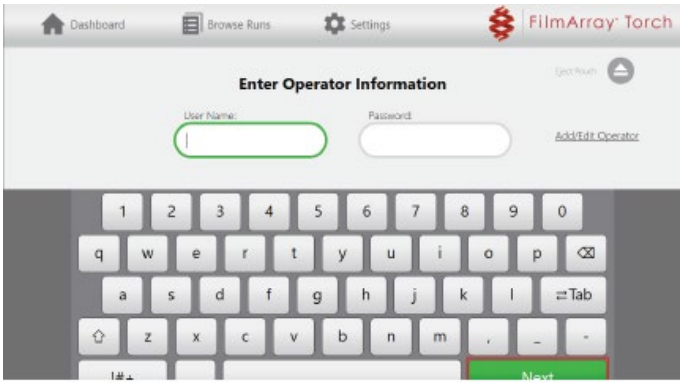
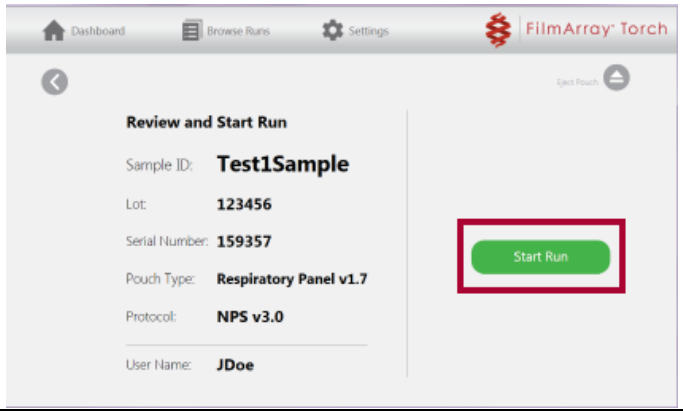
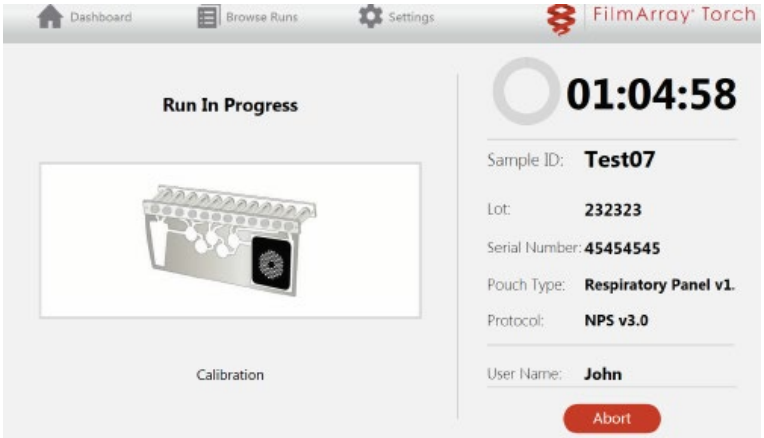
8.1	Specimen / Reagent Preparation
Preventing amplicon contamination • Discard used pouches in a biohazard container immediately after the run has completed. • Avoid excessive handling of pouches after test runs. • Avoid exposing pouches to sharp edges or anything that may cause a puncture. • Use clean gloves to remove materials from bulk packaging bags, and reseal bulk packaging bags when not in use.	
Prepare Pouch	
1	Thoroughly clean the work area and the Pouch Loading Station with freshly prepared 10% bleach (or suitable disinfectant) followed by a water rinse. This MUST be completed before and after loading pouches for testing and between each patient.
2	Don clean gloves. Remove the pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective aluminum canister.
3	Check the expiration date on the pouch. Do not use expired pouches.
4	Slide the pouch into the Pouch Loading Station, aligning the red and blue labels on the pouch with the red and blue arrows on the Pouch Loading Station. 
5	
6	Place a blue-capped Hydration Injection Vial into the blue well of the Pouch Loading Station.
7	Place a red-capped Sample Injection Vial into the red well of the Pouch Loading Station.
Hydrate Pouch	
8	Twist and lift the Hydration Injection Vial, leaving blue cap in the well of the Pouch Loading Station.

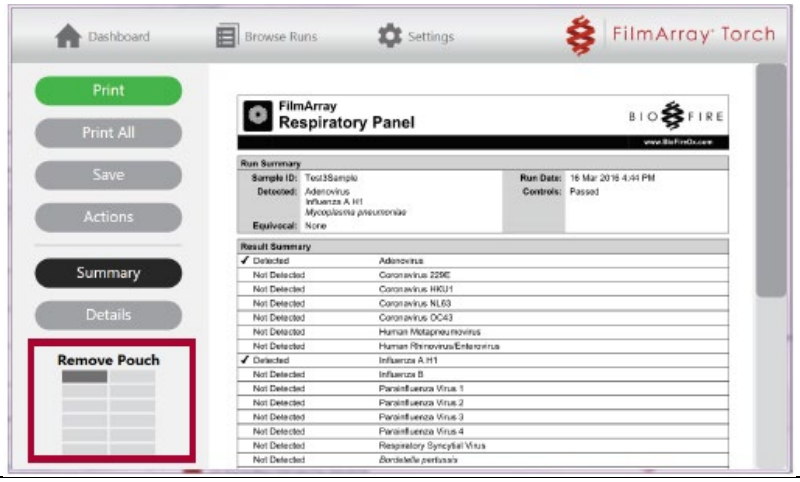
8.1	Specimen / Reagent Preparation
9	Insert the cannula tip into the port in the pouch located directly below the blue arrow of the Pouch Loading Station. Push down forcefully in a firm and quick motion until you hear a faint "pop" and feel an ease in resistance. The correct volume of liquid will be pulled into the pouch by vacuum. 
10	Verify that the pouch has been hydrated.
11	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	
12	Hold the Sample Buffer ampoule so that the tip is facing up.
13	Pinch the textured plastic tab on the side of the ampoule until the seal snaps. 
14	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. **Avoid squeezing the ampoule additional times as this will generate excessive bubbles.** 
15	Thoroughly mix the patient sample.

8.1	Specimen / Reagent Preparation
16	Use the transfer pipette provided in the test kit to draw CSF sample to the second line (approximately 0.2 mL) of the Transfer Pipette. Add the CSF sample to the Sample Buffer in the Sample Injection Vial. Discard the Transfer Pipette into a biohazard waste container.  **Note: DO NOT use the Transfer Pipette to mix the sample once it is loaded into the Sample Injection Vial.**
17	Remove the Sample Injection Vial from the Pouch Loading Station and gently invert the vial at least three times to mix. 
18	Return the Sample Injection Vial to the red well of the Pouch Loading Station.
Load Sample Mix	
19	Slowly twist the Sample Injection Vial so it loosens from its red cap and **pause for 5 seconds.** **NOTE:** Waiting 5 seconds decreased the risk of dripping and contamination from the sample.  Lift the Sample Injection Vial, leaving the red cap in the well of the Pouch Loading Station.
20	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.

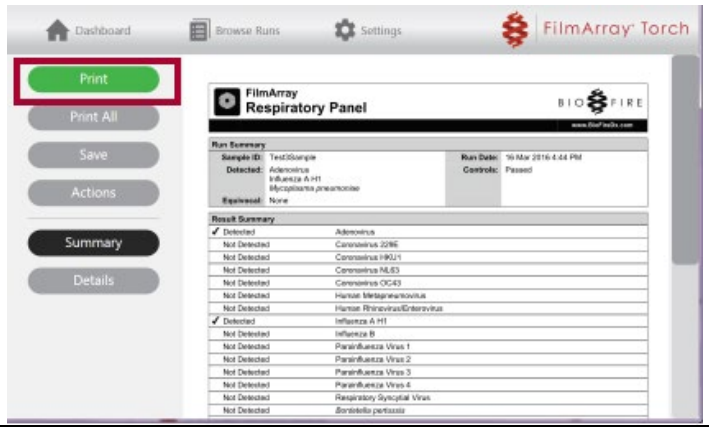
8.1	Specimen / Reagent Preparation
21	Forcefully push down in a firm and quick motion to puncture seal (a faint "pop" is heard) and sample is pulled into the pouch by vacuum.
22	Verify that the sample has been loaded. Flip the barcode label down and check to see that fluid has entered the reagent well 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
23	Discard the Sample Injection Vial and the Hydration Injection Vial in an appropriate biohazard sharps container.
24	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.
25	Discard gloves.

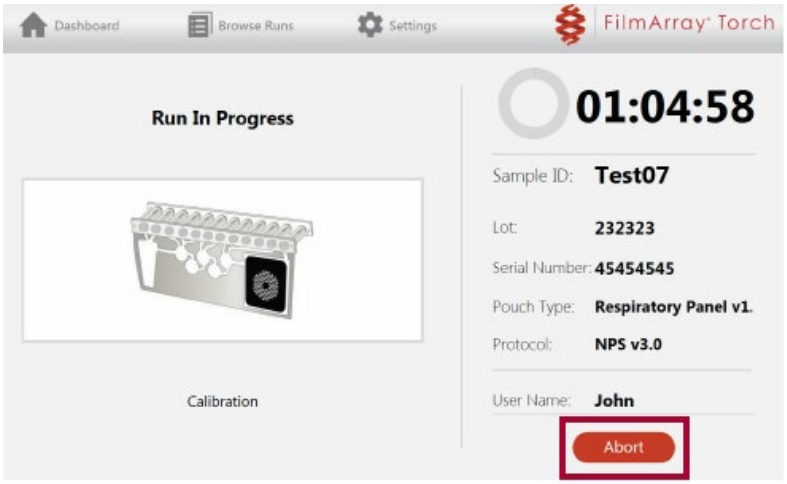
8.2	Run Pouch - BioFire® FilmArray® Torch
1	Ensure the BioFire® FilmArray® system (instrument and computer) is powered on and the software is launched.
2	Don clean gloves. Select an available Module on the touch screen.
3	Scan the pouch barcode on the fitment label. Pouch identification (Lot Number and Serial Number), Pouch Type, and Protocol are preprogrammed in the rectangular barcode located on the 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.
4	Scan the barcoded Sample label to enter the Sample ID. The Sample ID can be entered manually if the barcode scanner is not functioning properly.
5	Insert the pouch into the module. Ensure 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. 

8.2	Run Pouch - BioFire® FilmArray® Torch
6	Enter operator username and password, then select **Next**.  **NOTE**: The font color of the username is red until the username is recognized by the software.
7	Review the entered run information on the screen. If correct, select **Start Run**. 
8	Once the run has been started, the selected Module's LED will turn solid green to indicate that 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. The operator may navigate to the Dashboard to perform other tasks. 

8.2	Run Pouch - BioFire® FilmArray® Torch
9	At the end of the run, the Dashboard changes the status of the Module to **Finished** and the pouch is partially ejected. Do not remove the pouch until after the next step. One copy of the report will print automatically.
10	To finish the run, select the Finished Module on the Dashboard to view the report.
11	Remove the pouch from the Module and immediately discard the pouch in a biohazard container. The Module LED is solid blue indicating that the Module is ready for a new run. **NOTE: Once the pouch has been removed, the report can only be viewed through the Browse Runs feature.** 

8.3	Viewing and Printing Reports
1.	When a run is finished, the report can be viewed on the: • Run in Progress screen – the run report displays once the run is complete. • Dashboard screen – a report icon appears and the status changes to Finished. Selecting the Module box displays the run report. Once the pouch is removed from the BioFire Torch Module, the status changes to Available. • Browse Runs screen – the run reports are accessible from the table

8.3	Viewing and Printing Reports
2.	To print a report from a previous BioFire® Pouch run: • Select Browse Runs in the top menu on the touch screen. • Select one desired run from the table. NOTE: If no runs or multiple runs are selected, the View Report option is disabled. • Select View Report to pen the report page.  • Select Print 

8.4	Special Handling
1.	Abort Run: If a run needs to be stopped before it is finished, select the applicable BioFire® Torch Module from the Dashboard. The Run In Progress screen will display the current run information; select Abort. Any data that has been generated for the aborted run will not be available for analysis. An aborted run cannot be restarted and the pouch mut not be re-run.  NOTE: Aborting a run may take up to five minutes to complete.
2.	Error Messages: If errors occur, see Chapter 8, Preventative Maintenance and Troubleshooting in the BioFire® FilmArray® Torch Operator's Manual, for more information on viewing and handling error messages.

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

9. CALCULATIONS

Not applicable

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

The FilmArray software automatically analyzes and interprets assay results and displays the final results in a test report. When the 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 appropriate 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 and compares it 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 *N. 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 infection. Detection of these viruses may indicate primary infection, secondary reactivation, or the presence of latent virus. Results should always be interpreted I conjunction with other clinical, laboratory, and epidemiological information.

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.

Control Result	Explanation	Action Required	Outcome
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.

Table 2. Reporting of Results and Required Actions

Assay Result Reported	Interpretation of Result	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.

Assay Result Reported	Interpretation of Result	Action
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/ Failed	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 2, Interpretation of Controls Field on FilmArray Report, for instruction.

10.2 Rounding
Not applicable

10.3 Units of Measure
Not applicable

10.4 Analytical Measurement Range (AMR)
Not applicable

10.5 Review Patient Data

- Review patient results for unusual patterns, trends or distribution.
- Report atypical or unexpected results or trends for this test to appropriate supervisory personnel, prior to releasing results.
- Unusual findings must be investigated, and records of all data evaluation must be documented, including corrective action as needed.

10.6 Repeat Criteria and Resulting

IF the result is …	THEN…
Error/No Result/Invalid/Failed	Repeat testing
Remains unresolved following repeat testing	Cancel using the appropriate message
Control result: Passed	None – report the results provided on the test report
Viruses, Bacteria, and Yeast	
Detected[a]	Detected
Not Detected	Not Detected

IF the result is …	THEN…
Equivocal	Retest the original specimen using a new pouch and report the results of the retest.
Invalid	Retest the original specimen using a new pouch and report the results of the retest.

[a]If multiple organisms are detected in a specimen, retesting is required to confirm the polymicrobial result.

Message Code	Message
Detected	DET
Not Detected	NTD

Replacement of Failed Pouches

BioFire will replace failed pouches. We must document the following information on the BioFire Failed Pouches for Credit Log and submit to BioFire in order to be reimbursed.

Date
Initials
Assay – RP2.1, BCID, or ME
Instrument S/N - SGMC-TB010179, WOMC-TB010182
Pouch Lot #
Error Categories - Hydration Failure, Loss of Vacuum, Control Failure, Software Error, Instrument Error, Others
Comments - in comments it is important to put any code number for the errors – software and instrument. And any further information if you are reporting an "others" error.

To Look up an error code

1. Select Browse Runs in the top menu
2. Use the search icon to search for runs
3. Select a single run from the table
4. Select View Report
5. Click on actions
6. Select show run details
7. Look for an Error code

Result Reporting

- BioFire is interfaced with Sunquest to upload results. See addendum 1.

11. EXPECTED VALUES

11.1 Reference Intervals

Not detected

11.2 Critical Values

Detected

11.3 Standard Required Messages

Message Code	Result Always Message(s) for test BFMEC
MECOM	Results should be interpreted in the context of other diagnostic methods and clinical presentation.

The following message is added to the report when both HSV-1 and HSV-2 are Not Detected.

Message Code	Additional Message(s) for test BFHSCC
MEHS	Testing of spinal fluid for HERPES SIMPLEX VIRUS 1 & 2 DNA REAL TIME PCR QUEST is suggested if clinical suspicion of HSV infection is high.

The following message is added to the report when *Cryptococcus neoformans/gattii* is Not Detected.

Message Code	Additional Message(s) appended to CRYNG
MECN	Cryptococcal antigen testing of spinal fluid is suggested if clinical suspicion of cryptococcal infection is high.

The following message is added to the report when CMV is Detected.

Message Code	Additional Message(s) appended to CCMV
CMVC	WARNING: The FilmArray ME Panel does not distinguish between latent and active CMV. Detection of this virus 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.

The following message is added to the report when HHV-6 is Detected.

Message Code	Additional Message(s) appended to CHH6
CHH6C	WARNING: The FilmArray ME Panel does not distinguish between latent and active HHV-6 infections. Detection of this virus 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.

12. CLINICAL SIGNIFICANCE

Central nervous system (CNS) infections are responsible for causing inflammatory conditions of the brain and/or meningeal tissues surrounding the brain (i.e., meningitis, encephalitis, meningoencephalitis; here collectively termed ME). Approximately 15% of cases are fatal and many other cases result in life-long disabilities such as loss of limbs, visual and hearing deficits, seizures, and altered learning and memory. The FilmArray ME panel conducts tests for the identification of 14 potential CNS pathogens from CSF. The specimen can be tested using the FilmArray ME Panel with results available within about one hour.

Bacteria	Viruses	Yeast
• *Escherichia coli* K1 • *Haemophilus influenzae* • *Listeria monocytogenes* • *Neisseria meningitidis* (encapsulated) • *Streptococcus agalactiae* • *Streptococcus pneumoniae*	• Cytomegalovirus (CMV) • Enterovirus (EV) • Herpes simplex virus 1 (HSV1) • Herpes simplex virus 2 (HSV2) • Human herpesvirus 6 (HHV6) • Human parechovirus (HPeV) • Varicella zoster virus (VZV)	• *Cryptococcus neoformans/gattii*

13. PROCEDURE NOTES

- **FDA Status: FDA Exempt/Cleared or Approved**
- **Validated Test Modifications:** None

14. LIMITATIONS OF METHOD

- False negative results may occur when the concentration of organism(s) in the specimen is below the device limit of detection.
- Due to the small number of positive prospective and retrospective specimens for certain organisms, performance characteristics for *Escherichia coli, Haemophilus influenzae, Listeria monocytogenes, Neisseria meningitidis, Streptococcus agalactiae*, Cytomegalovirus, and Human parechovirus were established primarily using contrived clinical specimens.
- Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for HSV-1, HSV-2, Human parechovirus, Varicella, HHV-6, and *C. neoformans/gattii* were established with retrospective clinical specimens.
- This test is a qualitative test and does not provide a quantitative value for the organism(s) in the specimen.
- Results from this test must be correlated with clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- The performance of this test has not been established for CSF specimens from patients without signs and/or symptoms of meningitis and/or encephalitis.

- The performance of this test has not been specifically evaluated for CSF specimens from immunocompromised individuals.
- The effect of antibiotic treatment on test performance has not been evaluated.
- The performance of this test has not been established for monitoring treatment of infection with any of the panel organisms.
- **This test in not intended for use with CSF collected from indwelling medical devices (e.g., CSF shunts).**
- **CSF specimens should not be centrifuged prior to testing.**
- 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 of the Instruction Booklet could lead to erroneous results.
- A negative FilmArray ME Panel result does not exclude the possibility of CNS infection and should not be used as the sole basis for diagnosis, treatment, or other management decisions. There is a risk of false negative values due to the presence of sequence variants or rearrangements in the gene targets of the assay, procedural errors, inhibitors in specimens, technical error, sample mix-up, or infections caused by an organisms not detected by the FilmArray ME 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.
- The detection of organism nucleic acid is dependent upon proper sample collection, 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 PCR2 control will not indicate whether or not nucleic acid has been lost due to inadequate collection, transport, or storage of specimens.
- Positive and negative predictive values are highly dependent on prevalence. False positive results are more likely for low prevalence analytes.
- Viral, bacteria, and yeast nucleic acid may persist in vivo independently of organism viability. Detection of organism targets does not imply that the corresponding organisms are infectious or are the causative agents for clinical symptoms.
- HHV-6 or CMV can exist in latent form that is reactivated during infection due to other pathogens, including agents not detected by the FilmArray ME Panel that may cause meningitis/encephalitis (e.g., *Mycobacterium 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 settings and following expert consultation.
- Viral shedding into the CSF often occurs in cases of zoster (shingles; caused by reactivation of VZV). VZV may not be the cause of CNS disease in these cases.
- Organism and amplicon contamination may produce erroneous results for this test. Particular attention should be given to the laboratory precautions noted under the preventing organism and amplicon contamination sections.
- Some organisms detected by the FilmArray ME Panel, such as *S. pneumoniae* and *H. influenzae* can be shed from the respiratory tract of healthy individual. HSV-1 may also be shed from individuals with active or recurrent cold sores. Particular attention should be

given to the laboratory precautions noted under the preventing organism and amplicon contamination sections. Caution should also be exercised during specimen collection and testing to prevent contamination leading to false positives.

- If two or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.
- Cross-reactivity with organisms in addition to those listed in the Analytical Specificity section of the Instruction Booklet may lead to erroneous results. Cross-reactivity with human rhinoviruses may occur, but rhinoviruses are rarely present in CSF and are not a recognized cause of meningitis. Caution should be exercised during specimen collection and testing to prevent contamination with rhinovirus associated with respiratory infections.
- Only *E. coli* strains possessing the K1 capsular antigen will be detected. All other E. coli strains/serotypes will not be detected.
- Only encapsulated strains of *N. meningitidis* will be detected. Unencapsulated *N. meningitidis* will not be detected.

14.1 Precision

Refer to the FilmArray Meningitis/Encephalitis Panel Instruction Booklet.

14.2 Interfering Substances

Potentially interfering substances that could be present in CSF specimens or introduced during specimen collection and testing were evaluated for their effect on FilmArray ME Panel performance. Each substance was added to contrived samples containing representative ME Panel organisms at concentrations equivalent to approximate 3xLOD. The concentration of substance added to the samples was equal to or greater than the highest level expected to be in CSF specimens (based on reference concentrations for normal or meningitis/encephalitis CSF, as indicated in Table 25 of the package insert).

The majority of substances evaluated has no effect on the FilmArray ME Panel control assays or organism test results. Valid results were obtained and each organism was detected in samples containing relevant and/or elevated levels of endogenous substances such as lactate, glucose, proteins (≤ 15mg/mL), white blood cells, human genomic DNA, and blood, in samples added to transport media, and in samples containing ethanol (see Table 25 of the FilmArray Meningitis/Encephalitis Panel Instruction Booklet). Interference or damage to the sample was observed with high levels of protein (albumin >15mg/mL) or with bleach at a concentration >0.1% (v/v).

14.3 Clinical Sensitivity/Specificity/Predictive Values

Refer to the FilmArray Meningitis/Encephalitis Panel Instruction Booklet.

15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

16. RELATED DOCUMENTS

- FilmArray® Meningitis/Encephalitis (ME) Panel Instruction Booklet RFIT-PRT-0276-02 May 2016, BioFire Diagnostics, LLC.
- BioFire FilmArray-Torch Operator's Manual, BioFire Diagnostics, LLC.
- BioFire® FilmArray® Torch Systems Maintenance procedure SGMC.M1019
- BioFire Failed Pouches for Credit Log (AG.F565)
- FilmArray Torch Maintenance Record (AG.F516)
- BioFire FilmArray Meningitis/Encephalitis Panel (ME) External QC Form (AG.F640)
- BioFire FilmArray Meningitis/Encephalitis Panel (ME) Internal QC Log (AG.F639)

17. REFERENCES

1. FilmArray® Meningitis/Encephalitis (ME) Panel Instruction Booklet RFIT-PRT-0276-02 May 2016, BioFire Diagnostics, LLC.
2. Centers for Disease Control and Prevention (CDC). Emergence of Cryptococcus gattii--Pacific Northwest, 2004-2010. MMWR Morb. Mortal. Wkly. Rep. 59, 865–868 (2010).
3. Duff S, Hasbun R, Ginocchio CC, Balada-Llasat JM, Zimmer L, Bozzette SA. Economic analysis of rapid multiplex polymerase chain reaction testing for meningitis/encephalitis in pediatric patients. Future microbiology. 2018; 9;13(06):617-29.
4. Hanson EK. The First Fully Automated Molecular Diagnostic Panel for Meningitis and Encephalitis: How Well Does It Perform, and When Should It Be Used? JCM 2016; 54 (9):2222-2224.
5. Leber AL *et al*., Multicenter Evaluation of BioFire FilmArray Meningitis/Encephalitis Panel for Detection of Bacteria, Viruses, and Yeast in Cerebrospinal Fluid Specimens JCM 2016; 54 (9): 2251-2261.
6. Liesman RM. Strasburg AP, Heitman AK, Theel ES, Patel R, Matthew J. Binnickera MJ Evaluation of a Commercial Multiplex Molecular Panel for Diagnosis of Infectious Meningitis and Encephalitis JCM 2018; 56 (4) e01927-17.
7. van de Beek *et al*., Diagnosis and treatment of acute bacterial meningitis. Clin Microbiol Infect 2016; 22: S37–S62.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

Addendum 1:

Result Reporting

General information:

- The BioFire is interfaced with Sunquest.
- Upon completion of testing, the results will print dynamically to the BioFire printer and transmit to Sunquest. **Review Instrument Printout**
    - If ALL results are negative then the results will autofile into Sunquest and transmit to Cerner.
    - If any of the tests are Positive, then ALL the tests for that accession number are held in Sunquest. (Refer to section 10.6 if multiple results are positive and retest as needed)
- Review results in Sunquest OEM

Reviewing and releasing results:

1. Access OEM
   At DEVICE: prompt, type in Method code SGMC: SGBF
   WOMC: WOBF
2. Results will display cup by cup.
   - If ALL are negative, then those results auto-filed and require no action. Proceed to next cup.
   - For positive results that were held, continue with step 3 below.
   - Refer to *OEM - On Line Result Entry Method* procedure (LIS SOP) for additional information about review and release of results.
3. Positive (Detected) Results
   a. Positive results will be tagged with CALL in Sunquest to indicate the result must be called and documented using proper format.
   Append CBACK documentation to results including who you called, date, time and tech code; then click Accept to release. Required format is:

   -CBACK-;full name of person called DATE TIME Tech code
   Example -CBACK-;Sue Smith 032420 1420 4568

4. Perform an OFC (Online File Cleanup) at least once per shift. This process cleans up the online data that was sent to Sunquest.
   a. In Sunquest (SmarTerm) access function OFC
   b. Type in the method code (WOBF or SGBF).
   c. At the Start at Cup Number prompt, type in 1 and then press ENTER.
   d. At the Stop at Cup Number prompt, press ENTER.