### BioFire Blood Culture Identification Panel Testing

### Purpose

This procedure provides instructions for testing positive blood culture samples using the BioFire® FilmArray® Blood Culture Identification (BCID) Panel Kit.

### Background

The BioFire BCID Panel is a multiplexed nucleic acid test intended for use with the BioFire® FilmArray® 1.5, BioFire® FilmArray® 2.0, or BioFire® FilmArray® Torch Systems for the simultaneous qualitative detection and identification of multiple bacterial and fungal nucleic acids in positive blood culture samples.

The following gram-positive bacteria, gram-negative bacteria, and yeast are identified using the BioFire BCID Panel:

**Gram-positive bacteria**

* *Enterococcus*
* *Listeria monocytogenes*
* *Staphylococcus*
* *Staphylococcus aureus*
* *Streptococcus*
* *Streptococcus agalactiae*
* *Streptococcus pneumoniae*
* *Streptococcus pyogenes*

**Gram-negative bacteria**

* *Acinetobacter baumannii*
* *Enterobacteriaceae*
* *Enterobacter cloacae* complex
* *Escherichia coli*
* *Klebsiella oxytoca*
* *Klebsiella pneumoniae*
* *Serratia marcescens*
* *Proteus*
* *Haemophilus influenza*
* *Neisseria meningitidis* (encapsulated)
* *Pseudomonas aeruginosa*

**Yeast**

* *Candida albicans*
* *Candida glabrata*
* *Candida krusei*
* *Candida parapsilosis*
* *Candida tropicalis*

**Antimicrobial resistance genes**

* *mecA* – methicillin resistance
* *vanA/B –* vancomycin resistance
* KPC – carbapenem resistance

The BioFire® FilmArray® Blood Culture Identification (BCID) Panel also contains assays for the detection of genetic determinants of resistance to methicillin (*mecA*), vancomycin (*vanA* and *vanB*), and carbapenems (KPC) to aid in the identification of potentially antimicrobial resistant organisms in positive blood culture samples.

### Principle of the Procedure

The BioFire BCID Panel pouch is a closed system disposable that houses all the chemistry required to isolate, amplify, and detect nucleic acid from multiple bloodstream pathogens within a single blood culture sample. The rigid plastic component (fitment) of the BioFire BCID Panel 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 BioFire BCID Panel loads the sample into the BioFire BCID Panel pouch, places the pouch into the BioFire® 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 pouch 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 10 minutes, and the bead-beater apparatus can be heard as a high-pitched whine during the first minute of operation.
2. 1st Stage Multiplex PCR—The purified nucleic acid solution is combined with a preheated master mix to initiate 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 first-stage PCR are diluted and mixed with fresh PCR reagents containing a double-stranded DNA binding dye (LCGreen® Plus, BioFire Diagnostics, LLC). This solution is distributed over the 2nd stage PCR array. The individual wells of the array contain primers for different assays (each present in triplicate) that target specific nucleic acid sequences from each of the pathogens detected, as well as control template material. These primers are “nested,” or internal to the specific products of the 1st stage multiplex reaction, which enhances both the sensitivity and specificity of the reactions.
4. DNA Melting Analysis—After 2nd stage PCR, the temperature is slowly increased, and fluorescence in each well of the array is monitored and analyzed to generate a melt curve. The temperature at which a specific PCR product melts (melting temperature or Tm) is consistent and predictable, and the BioFire® 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.

### Sample Requirements

The following table describes the requirements for specimen collection, preparation, and handling that will help ensure accurate test results.

|  |  |
| --- | --- |
| **Sample Type** | Blood culture samples identified as positive by a continuous monitoring blood culture system. Results are intended to be interpreted in conjunction with Gram stain results. |
| **Sample Collection** | Sample should be collected from the blood culture bottle using a syringe that is capable of measuring 0.2 mL. |
| **Sample Volume** | 0.2 mL (200 µL) |
| **Sample Age and Storage** | Blood culture samples should be processed and tested as soon as possible after being flagged as positive by the culture instrument. If storage is required, specimens can be held:   * At room temperature for up to 8 hours (15-25 °C) * In the culture instrument prior to testing |

### Materials

|  |  |
| --- | --- |
| **Materials Provided** | **Materials Required But Not Provided** |
| Each kit contains sufficient reagents to test 30 or 6 specimens:   * Individually packaged BioFire® FilmArray® Blood Culture Identification (BCID) 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) * Individually packaged Transfer Pipettes | BioFire® FilmArray® System including:   * BioFire® FilmArray® 1.5, BioFire® FilmArray® 2.0, or BioFire® FilmArray® Torch Systems * BioFire FilmArray Software * Pouch Loading Station compatible with the use of the Injection Vials |

### Quality Control

**Process Controls**

Two process controls are included in each pouch:

1. **DNA Process Control**

The DNA Process Control assay targets DNA from the yeast *Schizosaccharomyces pombe*. The yeast is present in the pouch in a freeze-dried form and is hydrated and introduced into the test when the sample is loaded. The control material is carried through all stages of the test process, including lysis, nucleic acid purification, 1st stage PCR, dilution, 2nd stage PCR, and DNA melting. A positive control result indicates that all steps carried out in the pouch were successful.

1. **PCR2 Control**

The PCR2 Control assay detects a DNA target that is dried into the 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 BioFire® FilmArray® Software will automatically fail the run if the melting temperature (Tm) for either the DNA Process Control or the PCR2 Control is outside an acceptable range (77.6–81.6 for the DNA Process Control and 74.2–78.2 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, RP2, GI, ME, and BCID) are used on the same BioFire® FilmArray® Instrument.

Good laboratory practice recommends running external positive and negative controls regularly. Uninnoculated blood culture media can be used as an external negative control. Previously characterized positive blood culture samples or 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 BioFire® FilmArray® Blood Culture Identification (BCID) Panel Quick Guide for more details and pictorial representations of these instructions.

Gloves and other Personal Protective Equipment (PPE) should be used when handling pouches and samples. Only one BioFire BCID Panel pouch should be prepared at a time. Once the sample is added to the pouch, it should be promptly transferred to the BioFire® FilmArray® Instrument to start the run. After the run is complete, the pouch should be discarded in a biohazard container.

Step 1: 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.
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 Pouch Loading Station so that the red and blue labels on the pouch align with the red and blue arrows on the Pouch Loading Station.
2. Place a blue-capped Hydration Injection Vial in the blue well of the Pouch Loading Station.
3. Place a red-capped Sample Injection Vial in the red well of the Pouch Loading Station.

Step 2: Hydrate Pouch

1. Twist and lift the Hydration Injection Vial, leaving blue cap in the well of the Pouch Loading Station.
2. 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.
3. Verify that the pouch has been hydrated.
4. 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.

Step 3: 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 the side of the ampoule until the seal snaps.
2. 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.
3. Invert the positive blood culture bottle several times to mix.
4. Wipe the bottle septum with alcohol and air dry.
5. Tilt the bottle and hold in the tilted position to allow the bottle resin to settle (approximately 10 seconds).
6. Using a syringe, withdraw 200 uL of blood culture sample through the bottle septum, taking care to avoid drawing resin beads into the sample, or the formation of bubbles.
7. Add sample directly to Sample Buffer in the Sample Injection Vial. Discard syringe in an appropriate biohazard sharps container and return the Sample Injection Vial to the Pouch Loading Station.
8. Alternately: Draw the desired amount of blood culture sample (>200 uL) from the bottle into the syringe and transfer to a sterile secondary container. Draw the blood culture sample from the secondary container to the second line of the Transfer Pipette (200 uL) and add the sample to Sample Buffer in the Sample Injection Vial. Tightly close the lid of the Sample Injection Vial.

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

1. Remove the Sample Injection Vial from the Pouch Loading Station and gently invert the vial at least three times to mix.
2. Return the Sample Injection Vial to the Pouch Loading Station.

Step 4: 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 Pouch Loading Station.

1. 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 the Prepare Pouch section.
3. Discard the Sample Injection Vial and the Hydration Injection Vial in an appropriate biohazard sharps container.
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.

Step 5: Run Pouch

The BioFire® FilmArray® Software includes step-by-step instructions that guide the operator through performing a run. Brief instructions for BioFire® FilmArray® 1.5, BioFire® FilmArray® 2.0, and BioFire® FilmArray® Torch systems are given below. Refer to the appropriate BioFire® FilmArray® Operator’s Manual for more detailed instructions.

BioFire® FilmArray® Instrument(s)

1. Ensure that the computer and the BioFire Instrument(s) are on and the BioFireSoftware is launched.
2. Open the lid of an available instrument (if not already open).

**NOTE: An available instrument is indicated by a constant green light on the front of the instrument.**

1. Insert the pouch into the instrument.
2. Position the pouch so that the array is on the right with the film directed downward into the BioFire Instrument. The red and blue labels on the pouch should align with the red and blue arrows on the BioFire 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 pouch using the barcode scanner.
2. Pouch identification (Lot Number and Serial Number), Pouch Type, and Protocol are preprogrammed in the rectangular barcode located on the pouch, and 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.

**NOTE: The barcode cannot be scanned prior to placing the pouch in the instrument.**

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.
2. If necessary, select a protocol from the Protocol drop-down list (the protocol is usually selected automatically).
3. Enter a user name and password in the Name and Password fields.
4. Close the instrument lid.
5. Click the Start Run button on the screen.
6. 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, follow the on-screen instructions to open the instrument and remove the pouch.
2. Immediately discard the pouch in a biohazard container.
3. Results are automatically displayed in the report section of the screen. The run file is automatically saved in the database and the report can be printed and/or saved as a PDF file.

BioFire® FilmArray® Torch

1. Ensure that the BioFire Torch system is on.
2. Select an available Module on the touch screen.
3. Scan the barcode on the pouch using the barcode scanner.

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.

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.
2. Insert the pouch into the Module.

Ensure that the pouch fitment label is lying flat on top of pouch and not folded over. As the pouch is inserted, the Module will grab onto the pouch and pull it into the chamber.

1. If necessary, select and/or confirm a protocol from the protocol drop-down list.
2. Enter operator user name and password, then select Next.

**NOTE: The font color of the username is red until the user name is recognized by the software.**

1. Review the entered run information on the screen. If correct, select 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. At the end of the run, the status of the Module changes to Finished and the pouch is partially ejected.
2. Select the Finished Module on the Dashboard to view the report.

Select Print to print the report, or Save to save the report as a file.

1. Remove the pouch from the Module and immediately discard the pouch in a biohazard container.

**NOTE: Once the pouch has been removed, the report can only be viewed through the Browse Runs feature.**

### Interpretation

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

**Assay Interpretation**

When 2nd stage PCR is complete, the BioFire® 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 the BioFire® FilmArray® Operator’s Manual). The BioFire® FilmArray® Software then performs several analyses and assigns a final assay result.

Analysis of melt curves. The BioFire 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**

Interpretations for many of the organisms are based on the results of a single assay. Interpretations for *Haemophilus influenzae* and the *Staphylococcus, Streptococcus*, and *Enterobacteriaceae* groups rely on the results of several assays. The antimicrobial resistance genes are also based on the result of a single assay; however, the test results are only reported when specific organisms are also detected in the same sample. Interpretation guidelines for actions to be taken based on the test result are described in the FilmArray Blood Culture Identification Panel Instruction Booklet. The FilmArray Blood Culture Identification Booklet contains information about known assay limitations (e.g., cross-reactivity, strains that are not detected) that may be important in the interpretation of the test result and in correlating the results with the result of standard culture and biochemical identification.

NOTE: Polymicrobial blood cultures with 3 or more distinct organisms are possible but rare. If Detected results are reported for 3 or more organisms in a sample, a retest of the sample is recommended to confirm the polymicrobial result.

NOTE: In some cases, the Gram stain result and results from the FilmArray BCID Panel may be discrepant (for example, detection of gram-positive cocci by the FilmArray BCID Panel when gram-positive cocci are not observed in the Gram stain). In these cases, the FilmArray BCID Panel results should be confirmed (e.g. by culture) before reporting, unless the result is concordant with other laboratory, epidemiological, or clinical findings.

**BioFire BCID Panel Test Report**

The BioFire BCID Panel test report is automatically displayed upon completion of a run and contains three sections: the Run Summary, the Results Summary, and the Run Details (see the BioFire Blood Culture Identification 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, then None will be displayed in the Detected field. Antimicrobial resistance genes with a result of Detected or Not Detected will be listed in the corresponding field of the summary. 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 **Results Summary—Interpretations** 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. Possible results for antimicrobial resistance genes are Detected, Not Detected, 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 (DNA 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 (0 or 1 positive replicates for either of the controls, each of which is tested in triplicate). 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.

The table below provides a summary and explanation of the possible control results and follow-up actions.

| **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 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. |

### 

### 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. The table below provides an explanation for each interpretation and any follow-up necessary to obtain a final result.

| **Result** | **Explanation** | **Action** |
| --- | --- | --- |
| Detected | The run was successfully completed  AND  The pouch controls were successful (Passed)  AND  The assay(s) for the organism (or antimicrobial resistance gene) were POSITIVE. | Report results.  NOTE: If Detected results are reported for 3 or more organisms in a sample, a retest of the sample 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) for the organism (or antimicrobial resistance gene) were NEGATIVE. | Report results. |
| Invalid | The pouch controls were not successful (Failed)  OR  The run did not complete successfully  (Run Status displayed as: Aborted, Incomplete, Instrument Error, Software Error, or Instrument Communication Error). | See Table 11 Interpretation of Controls Field on the Test Report for instruction. |
| N/A  (Antimicrobial Resistance Genes only) | The run was successfully completed  AND  The pouch controls were successful (Passed)  AND  The assay(s) for the organism(s) associated with the antimicrobial resistance gene were NEGATIVE so the results of the antimicrobial resistance gene are not applicable to the test results. | Report results. |

### References/Related Documents

FilmArray Blood Culture Identification Panel (BCID) Instruction Booklet (RFIT-PRT-0369), BioFire Diagnostics, LLC.