**PURPOSE:**

This document provides instructions for testing positive blood culture samples using the BioFire BCID2 Panel Kit.

**SCOPE:**

This procedure applies to Hanover Hospital laboratory

**PRINCIPLE:**

The BioFire BCID2 Panel is a multiplexed nucleic acid test intended for use with BioFire FilmArray Torch Systems for the simultaneous qualitative detection and identification of multiple bacterial and yeast nucleic acids and select genetic determinants associated with antimicrobial resistance. The BioFire BCID2 panel test is performed directly on blood culture samples identified as positive by continuous monitoring blood culture system. Results are intended to be interpreted in conjunction with Gram stain results.

The BioFIre BCID2 panel pouch is a closed system disposable that stores all the necessary reagents for sample preparation, polymerase chain raction (PCR) and detection in order to isolate, amplify and detect nucleic acid from multiple pathogens and antimicrobial resistance genes contained n blood culture samples identifyied as positive bya continuous monitoring blood culture system. After sample collection, the user injects hydration solution and sample combined with sample buffer into the pouch, places the pouch into a BioFire FilmArray Torch module and starts a run. The entire run process takes about an hour.

Additional details can be found in the BioFire FilmArray Tourch system operator's manual.

**REAGENTS/SUPPLIES:**

BioFire FilmArray Torch System

Pouch Loading Station

BioFire BCID2 Pouches

Single use sample buffer ampoules

Single use prefilled hydration injection vials (blue)

Single use sample injection vials (red)

Individually packaged transfer pipet

Syringe capable of measuring 0.2 ml

Saf-T Holder® device

Main Molecular Film Array BCID2 Control Panel M416

10% Bleach solution

**SAFETY PRECAUTIONS:**

1. Wear appropriate PPE, including (but not limited to) disposable clean powder free gloves and lab coats. Protect skin, eyes and mucous membranes. Chanage gloves often when handling reagents or samples.
2. Follow standard precautions when performing the test.
3. **WARNING: Never add Bleach to Sample Buffer or sample waste.** Sample buffer will form hazardous fumes and compounds when mixed with bleach or other disinfectants.

**QUALITY CONTROL PLAN:**

**Process Controls**

Two process controls are included in each pouch: Both control assays must be positive for the test run to pass. If the controls fail, the sample should be retested using a new 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.

**2. 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 samples must be positive for the test run to pass. If the controls fail, the sample should be retested using a new pouch.

**Monitoring Test System Performance**

The FilmArray software will automatically fail the run if the melting temperature (Tm) for either the DNA Process Control or the PCR2 Control is outside an acceptable range (77.4-81.4 for the DNA Process Control and 73.8 - 77.8 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. Refer to the BioFire FilmArray Torch manual for instructions on obtaining control assay Tm values.

**External Controls**

**Note:** External controls are run daily until an IQCP has been completed an approved. After approval external controls are run as listed below:

* Each new lot or shipment
* At least every 30 days
* If storage temperature for the test kit or testing area is outside of 2- 30˚ C.
* More frequently if issues arise that dictate more frequent testing of controls.

**QC Procedure**

1. Allow controls to come to room temperature, approximately 30 minutes.
2. Use control as provided. **Do not dilute.**
3. Immediately before use, mix the control by inverting several times and vortexing for 3 - 5 seconds.
4. Tap the tube several times before opening.
5. Prepare sample mix, invert at least 3 times, load and run a BCID2 pouch. Follow the patient procedure below using 0.2 ml of the QC specimen.

**SPECIMEN**

Positive blood culture sample identified as positive by the BacTec FX and demonstrates the presence of organisms as determined by Gram stain.

## PROCEDURE

Clean gloves and a fluid resistant lab coat must be wornwhen handling pouches and samples. Prepare one BioFire BCID2 Panel pouch at a time and change gloves between samples and pouches. Once the sample is added to the pouch, promptly transfer the pouch to the instrument to start the run. After the run is complete, discard the pouch 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 canister.
3. Check the expiration date on the pouch. Do not use expired pouches.
4. Insert 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. Place a red-capped Sample Injection Vial into the red well of the Pouch Loading Station.
6. Place a blue-capped Hydration Injection Vial into the blue well of the Pouch Loading Station.

**Step 2: Hydrate pouch**

1. Unscrew the Hydration Injection Vial from the blue cap.
2. Remove the Hydration Injection Vial, leaving the blue cap in the Pouch Loading Station.
3. Insert the Hydration Injection Vial's cannula tip into the pouch hydration port located directly below the blue arrow of the Pouch Loading Station.
4. Forcefully push down in a firm and quick motion to puncture seal until a faint"pop" is heard and there is an ease in resistance. Wait as the correct volume of Hydration Solution is pulled into the pouch by vacuum.
   * If the hydration solution is not automatically drawn into the pouch, repeat Step 2 to verify that the seal of the pouch hydration port was broken. If hydration solution is again not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch.*
5. Verify that the pouch has been hydrated.
   * Flip the barcode label down and check to see that fluid has entered the reagent wells (located at the base of the rigid plastic part of the pouch). Small air bubbles may be seen.
   * If the pouch fails to hydrate (dry reagents appear as white pellets), repeat Step 2 to verify that the seal of the pouch hydration port was broken. If hydration solution is still not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch.*

**Step 3: Prepare Sample Mix**

1. Add Sample Buffer to the Sample Injection Vial.
   * Hold the Sample Buffer ampoule with the tip facing up.
   * Firmly pinch at textured plastic tab on the side of the ampoule until the seal snaps.
   * Invert the ampoule over the red-capped Sample Injection Vial and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.
2. Thoroughly mix the positive blood culture bottle by inverting it several times.
3. Wipe the bottle septum with alcohol and air dry.
4. Using a syringe and a Saf-T Holder® device, withdraw 0.2 ml of blood culture sample through the bottle septum, taking care to avoid the formation of bubbles.
5. Add sample directly to Sample Buffer in the Sample Injection Vial. Discard syringe in an appropriate biohazard sharps container and tightly close the lid of the Sample Injection Vial.

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

1. Remove the Sample Injection Vial from the Pouch Loading Station and invert the vial at least 3 times

to mix.

1. Return the Sample Injection Vial to the red well of the Pouch Loading Station.

**Step 4: Load Sample Mix**

1. Slowly twist to unscrew the Sample Injection Vial from the red cap and wait for 5 seconds with the vial resting in the cap.
2. Lift the Sample Injection Vial, leaving red cap in the well of the Pouch Loading Station, and insert the Sample Injection Vial cannula tip into the pouch sample port located directly below the red arrow of the Pouch Loading Station.
3. 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.
4. Verify that the sample has been loaded.
   * Flip the barcode label down and check to see that fluid has entered the reagent well next to the sample loading port.
   * If the pouch fails to pull sample from the Sample Injection Vial, the pouch should be discarded. Retrieve a new pouch and repeat from *Step 1: Prepare Pouch.*
5. Discard the Sample Injection Vial and the Hydration Injection Vial in appropriate biohazard sharps container.
6. 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® Torch Software includes step-by-step, on-screen instructions that guide the operator through performing a run. Brief instructions for the BioFire®Torch system are given below. Refer to the appropriate BioFire® Torch System Operator's Manual for more detailed instructions.

1. Ensure that the BioFire Torch system is powered on.
2. Select an available module on the touch screen or scan the barcode on the pouch using the barcode scanner.
3. Pouch identification (Lot Number and Serial Number), Pouch Type and Protocol information will be automatically entered when the barcode is scanned. **Note:** 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: When selecting* a *Pouch Type manually, ensure that the Pouch Type matches the label on the BioFire BCID2 Panel pouch.***

1. Enter the Sample ID by scanning the patient label.
2. Insert the pouch into the available module.
   1. 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.
3. If necessary, select and/or confirm the appropriate protocol for your sample type from the Protocol drop down list. The BioFire BCID2 Panel has a single protocol available in the drop down list.
4. Enter a user name and password, then select **Next.**
5. 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.

1. At the end of the run, remove the partially ejected pouch, then immediately discard it in a biohazard waste container.
2. The run file is automatically saved in the BIoFIre Software database, and the test report can be viewed, printed and/or saved as a PDF file.

**Interpretation of Results**

Each positive and negative assay result is interpreted by the BioFire Software to provide results for the identification of specific bacteria, yeast, and antimicrobial resistance (AMR) genes.

For most species detected by the BioFire BCID2 Panel, the organism is reported as "Detected" if a single corresponding assay is positive. Results may also be reported for groups or complexes of closely related species *(Acinetobacter calcoaceticus-baumannii* complex, *Enterobacter cloacae* complex, and *Klebsiella pneumoniae* group), genera containing multiple clinically relevant species *(Proteus* spp., *Salmonella* spp., *Staphylococcus* spp., and *Streptococcus* spp.), and for a variety of species within multiple genera of the order *Enterobacterales.* Results for these groups are reported qualitatively as "Detected" or "Not Detected" based on one assay, or in some cases, multiple relevant assays. Reporting of AMR genes with one or more applicable bacteria also requires interpretation based on more than one assay result.

***NOTE: Polymicrobial blood cultures with four or more distinct organisms are possible but rare. If "Detected" results are reported for four 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 BioFire BCID2 Panel results may be discrepant (for example, detection of a gram-positive cocci by BioFire BCID2 Panel when gram­ positive cocci were not observed in the Gram stain). In these cases, the BioFire BCID2 Panel results should be confirmed (e.g. by culture) before reporting, unless the result is concordant with other laboratory, epidemiological, or clinical findings.***

***NOTE: Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for a genetic marker of antimicrobial resistance does not indicate susceptibility to associated antimicrobial drugs or drug classes. A Detected result for a genetic marker of antimicrobial resistance cannot be definitively linked to the microorganism(s) detected. Culture is required to obtain isolates for antimicrobial susceptibility testing, and BioFire BCID2 Panel results should be used in conjunction with culture results for the determination of susceptibility or resistance.***

**Analytes Detected by the Biofire BCID2 Panel**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gram Positive Bacteria** | | | |
| *Enterococcus faecalis* | *Staphylococcus* spp. | *Streptococcus* spp. | |
| *Enterococcus faecium* | *Staphylococcus aureus* | *Streptococcus agalactiae* (Group B) | |
| *Listeria monocytogenes* | *Staphylococcus epidermidis* | *Streptococcus pneumoniae* | |
| *Staphylococcus lugdunensis* | | *Streptococcus pyogenes* (Group A) | |
| **Gram Negative Bacteria** | | | |
| *Acinetobacter calcoaceticus-baumannii* co mplex | | *Enterobacterales* | |
| *Bacteroides fragilis* | | *Enterobacter cloacae* complex | |
| *Haemophilus influenzae* | | *Escherichia coli* | |
| *Neisseria meningitidis* (encapsulated) | | *Klebsiella aerogenes* | |
| *Pseudomonas aeruginosa* | | *Klebsiella oxytoca* | |
| *Stenotrophomonas maltophilia* | | *Klebsiella pneumoniae* group | |
|  | | *Proteus* spp. | |
|  | | *Salmonella* spp. | |
|  | | *Serratia marcescens* | |
| **Yeast** | | | |
| *Candida albicans Candida auris*  *Candida glabrata* | *Candida krusei Candida parapsilosis*  *Candida tropicalis* | *Cryptococcus neoformanslgattii* | |
| **Antimicrobial Resistance Genes** | | | |
| CTX-M  IMP | KPC *mecAIC*  *mcr-18 mecAIC* and MREJ (MRSA) | NDM  OXA-48-like | *vanAIB*  VIM |

|  |  |
| --- | --- |
| **Gram Positive Target Detection and Interpretation** | |
| **Targets detected** | **Interpretation** |
| *Staphylococcus* spp. | Results suggests one or more *Staphylococcus* spp., not *S. aureus, S. epidermis, or S. lugdunensis* |
| *Staphylococcus aureus* mecA/C and MREJ | Detection of mecA/C & MREJ - Results suggest methicillin resistant *Staphylococcus aureus* (MRSA). |
| *Staphylococcus aureus* | Results suggest methicillin susceptible *Staphylococcus aureus* (MSSA). |
| *Staphylococcus epidermidis* | Results suggest methicillin susceptible *Staphylococcus epidermidis* (MSSE) |
| *Staphylococcus epidermidis* and mecA/C | Detection of mecA/C - Results suggest methicillin resistant *Staphylococcus epidermidis* (MRSE). |
| *Staphylococcus lugdunensis* | Results suggest *Staphylococccus lugdunensis* SUSCEPTIBLE to methicillin. |
| *Staphylococcus lugdunensis* and mecA/C | Detection of mecA/C - Results suggest *Staphylococcus lugdunensis* RESISTANT to methicillin. |
| *Staphylococcus epidermidis* and *Staphylococcus lugdunensis and mecA/C* | Detection of mecA/C - *Staphylococcus lugdunensis* AND *S. epidermidis* present. Results suggest one or both of these species is methicillin RESISTANT. |
| *Enterococcus faecium* | Results suggest vancomycin SUSCEPTIBLE *Enterococcus faecium* |
| *Enterococcus faecalis* | Results suggest vancomycin SUSCEPTIBLE *Enterococcus faecalis* |
| *Enterococcus faecalis* and vanA/B | Detection of vanA/B- Results suggest vancomycin RESISTANT *Enterococcus faecalis* (VRE). |
| *Enterococcus faecium* and vanA/B | Detection of vanA/B- Results suggest vancomycin RESISTANT *Enterococcus faecium* (VRE). |
| *Enterococcus faecium* and *Enterococcus faecalis* andvanA/B | Detection of vanA/B- *Enterococcus faecium* and *E. faecalis*  present. Results suggest one or both of these is vancomycin RESISTANT (VRE). Subculturing and sensitivity testing is required to determine the species with which the vanA/B is associated |
| *Streptococcus spp.* | Results suggest one or more *Streptococcus* species, not *S. agalactiae, S. pneumoniae, or S. pyogenes* |
| *S. pneumoniae* | Results suggest *Streptococcus pneumoniae* |
| *S. pyogenes* | Results suggest *Streptococcus pyogenes (group A Strep)* |
| *S. agalactiae* | Results suggest *Streptococcus agalactiae (group B Strep)* |
| *Listeria monocytogenes* | Results suggest *Listeria monocytogenes* |

|  |  |
| --- | --- |
| **Gram Negative Target Detection and Interpretation** | |
| **Targets detected** | **Interpretation** |
| *Acinetobacter calcoaceticus- baumannii complex* | Results suggest *Acinetobacter calcoaceticus- baumannii complex* |
| *Bacteroides fragilis* | Results suggest *Bacteroides fragilis* |
| *Enterobacterales* | Results suggest one or more species of *Enterobacterales* detected, not *E.cloacae, K.aerogenes, K.oxytoca, K.pneumoniae* group, *Proteus* spp., *Salmonella* spp., or *S. marcescens*. |
| *Enterobacter cloacae complex* | Results suggest *Enterobacter cloacae complex* |
| *Klebsiella aerogenes* | Results suggest *Klebsiella aerogenes* |
| *Escherichia coli* | Results suggest *Escherichia coli* |
| *Haemophilus influenzae* | Results suggest *Haemophilus influenza* |
| *Klebsiella oxytoca* | Results suggest *Klebsiella oxytoca* |
| *Klebsiella pneumoniae group* | Results suggest *Klebsiella pneumoniae* group |
| *Neisseria meningitidis* | Results suggest *Neisseria meningitidis* |
| *Pseudomonas aeruginosa* | Results suggest *Pseudomonas aeruginosa* |
| *Proteus spp.* | Results suggest One or more *Proteus* species |
| *Salmonella* | Results suggest *Salmonella* species |
| *Serratia marcescens* | Results suggest *Serratia marcescens* |
| *Stenotrophomonas maltophilia* | Results suggest *Stenotrophomonas maltophila* |
| NDM | An organism in this specimen produces an NDM carbapenemase which confers resistance to carbapenems. |
| IMP | An organism in this specimen produces an IMP  Carbapenemase which confers resistance to carbapenems. |
| VIM | An organism in this specimen produces a VIM carbapenemase which confers resistance to carbapenems. |
| OXA-48-like | An organism in this specimen produces an OXA carbapenemase which confers resistance to carbapenems. |
| KPC | An organism in this specimen produces a KPC  Carbapenemase which confers resistance to carbapenems. |
| CTX-M | An organism in this specimen an extended spectrum beta-lactamase (ESBL) which are resistant to Ceftriaxone. |
| mcr-1 | An organism in this specimen is colistin resistant |
| No-Targets Detected | None of the organisms targeted by this panel were detected, please follow-up on the final blood culture for growth of organisms not detected by the panel |

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| --- | --- |
| **Yeast Target Detection and Interpretation** | |
| **Targets detected** | **Interpretation** |
| *Candida albicans* | Results suggest *Candida albicans* |
| *Candida auris* | Results suggest *Candida auris* |
| *Candida glabrata* | Results suggest *Candida glabrata* |
| *Candida krusei* | Results suggest *Candida krusei* |
| *Candida parapsilosis* | Results suggest *Candida parapsilosis* |
| *Candida tropicalis* | Results suggest *Candida tropicalis* |
| *Crytococcus neoformans/gatti* | Results suggest *Crytococcus neoformans/gatti* |

**Table 1 Reporting of Results and Interpretation**

|  |  |  |
| --- | --- | --- |
| **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 |
| **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, or Software Error) | See Table 2 for instructions |
| **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 |

**Table 2 Interpretation of the Controls Field on the BCID2 Test Report**

|  |  |  |
| --- | --- | --- |
| Passed | The run was successfully completed  AND  Both pouch controls were successful. | Report the results provided on the test report |
| Failed | The run was successfully completed BUT  At least one of the pouch controls (DNA Process Control and/or PCR2 Control) failed. | Repeat the test using a new pouch.  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 appropriate BioFire® System Operator's Manual or contact Technical Support for further instruction.  Once the error is resolved, repeat the test on the same module or on a different module. |

**Result Summary**

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. Possible results for each antimicrobial resistance gene are Detected, Not detected, N/A, or Invalid. Table 2 provides an explanation for each interpretation and any follow-up necessary to obtain a final result.

**Instrument Maintenance:**

**Weekly**

* Wipe the instrument down with 10% bleach followed by a water wipe.
* Soak loading stations in bleach for 10 minutes followed by a water rinse.
* Restart the instrument

**Monthly**

* Check filters, clean or replace as needed
* Archive runs after 500 runs

**REFERENCES:**

BioFire Blood Culture Identification 2 (BCID@) Panel Testing. Instructions for Use (RFIT-PRT-0841-02. BioFire Diagnostics LLC.