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BioFire® Blood Culture Identification 2 Panel by Nucleic Acid Amplification

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The document describes the procedure for the use of the BioFire® Blood Culture Identification 2 Panel (BCID2). The procedure is to be performed by trained Laboratory staff.

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II. CLINICAL SIGNIFICANCE:

Bloodstream infections (BSIs) occur when pathogenic organisms (e.g., bacteria or yeast) enter the bloodstream and cause disease. These infections are commonly identified by the growth of the pathogenic organism in blood culture. BSIs can lead to the development of sepsis, defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Globally, over 30 million BSIs are estimated to occur each year, leading to approximately 19 million cases of sepsis and 5 million sepsis-related deaths. Timely diagnosis and administration of effective treatment can significantly reduce mortality, duration of hospital stays, and costs due to BSI and sepsis. The BioFire BCID2 Panel tests a single positive blood culture sample to simultaneously provide results for multiple organisms and organism groups that cause BSIs and genetic markers associated with antimicrobial resistance. Rapid identification of the organism(s) in the blood culture, along with information about antimicrobial resistance gene status for select microorganisms, may aid the physician in making appropriate treatment decisions. The following organism types, subtypes, and resistance genes are identified using the BCID2 Panel:

Gram Positive Bacteria		
Enterococcus faecalis *Enterococcus faecium* *Listeria monocytogenes*	*Staphylococcus sp.* *Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus lugdunesis*	*Streptococcus sp.* *Streptococcus agalactiae* (Group B) *Streptococcus pneumoniae* *Streptococcus pyogenes* (Group A)
Gram Negative Bacteria		
Acinetobacter calcoaceticus-baumannii complex *Bacteroides fragilis* *Haemophilus influenzae* *Neisseria meningitidis* (encapsulated) *Pseudomonas aeruginosa*		*Enterobacterales* *Enterobacter cloacae* complex *Escherichia coli* *Klebsiella aerogenes* *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 neoformans/gattii*

Antimicrobial Resistance Genes				
CTX-M IMP	KPC *mcr-1*	*mecA/C* *mecA/C* and MREJ (MRSA)	NDM OXA-48 like	*vanA/B* VIM

Detailed Explanation of Antimicrobial Resistance Genes	
CTX-M	A class A extended-spectrum β-lactamase (ESBL) that originated due to a mobilization of chromosomal genes (bla) from *Kluyvera spp*. and confers resistance to a broad spectrum of cephalosporins. This group of β-lactamases can be plasmid-borne, and the blaCTX-M gene may be found in multiple copies per cell within a variety of gram-negative hosts. Phylogenetic analyses of CTX-M describe five main lineages or phylogroups (CTX-M groups 1, 2, 8, 9, and 25) and over 200 types or variants. CTX-M ESBLs are predominantly found in the *Enterobacterales* family. However, they have also been reported in other non-enteric, gram-negative bacteria such as *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Acinetobacter baumannii*, *Vibrio spp.* and *Aeromonas spp.* Over the last decade, CTX-M enzymes have overtaken other ESBLs, including TEM and SHV ESBL variants, in prevalence.
IMP	β-lactamases are plasmid-borne metallo-β-lactamases (MBLs) belonging to Ambler class B1 MBLs. More than 80 distinct IMP types have been identified which have the potential to confer different levels of antibiotic resistance to broad-spectrum β-lactams like carbapenems, cephamycins, and oxymino cephalosporins. MBLs hydrolyze almost all βlactams, rendering ineffective products, resulting in bacterial resistance to this class of antibiotics. Carriage of a blaIMP gene has been detected in strains of *Serratia marcescens*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Escherichia coli*, and *Enterobacter cloacae.*
KPC	The *Klebsiella pneumoniae* carbapenemase gene (blaKPC or referred to here as KPC), confers resistance to the carbapenem class of β-lactams and currently is thought to be the most common and rapidly emerging carbapenemase in the United States. KPCs are frequently carried on mobile genetic elements with the potential to spread between organisms. Though originally isolated from *Klebsiella pneumoniae*, the gene has since disseminated to other genera/species including *Acinetobacter, Pseudomonas, Enterobacter, Serratia, Salmonella, Escherichia coli*, *Klebsiella oxytoca*, and other *Enterobacterales*. There are more than 40 known KPC variants that have been identified (named up to KPC-46). Carbapenem-Resistant Enterobacteriaceae (CRE) are increasingly important pathogens in the hospital setting.
mcr-1	The plasmid-borne mobilized genetic determinant mcr-1 is an emerging marker of

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	public health importance. It is associated with elevated MICs to colistin, a last-resort drug for some multidrug-resistant infections. To date, nine homologous mcr genes termed mcr-1 through mcr-9 have been identified in various *Enterobacterales*. pHNSHP45, the plasmid containing mcr-1, is capable of not only transferring between *E. coli* strains at a very high rate but also of transferring into other *Enterobacterales*. Mechanistically, mcr genes encode phosphoethanolamine transferases, which transfer a phosphoethanolamine residue to the bacterial lipid A and greatly reduces its affinity for colistin and related polymyxins.
mecA/C	Methicillin-resistant (MR) staphylococci are a serious concern in both hospital-acquired and community-acquired infections. Few options exist for the treatment of these infections because the bacteria are resistant to both natural and semi-synthetic β-lactam antibiotics (e.g. oxacillin/methicillin). The primary mechanism of methicillin resistance is through the acquisition of the *mecA* gene that encodes a penicillin-binding protein (PBP2a) that has a low affinity for β-lactams. The *mecA* gene is carried on a chromosomally integrated mobile genetic element called the staphylococcal cassette chromosome mec (SCCmec).
***mecA/C* and MREJ**	**(Methicillin Resistant *Staphylococcus aureus*-MRSA) -** The SCCmec cassette integrates into a specific region in the Staphylococcus genome. In *S. aureus*, this insertion creates MREJ (SCCmec right-extremity junction), and molecular identification of this junction region provides specific identification of an *S. aureus* that carries the SCCmec cassette. A combined molecular detection of mecA/C, MREJ, and *S. aureus* indicates MRSA. However, it is possible for S. aureus to carry SCCmec that has lost the mecA/C gene (an 'empty cassette'); such a strain would be a methicillin-susceptible *S. aureus* but could be misidentified by molecular methods if there is a co-detection of an additional Staphylococcus spp. that carries the mecA/C gene.
NDM	The New Delhi metallo-β-lactamase (NDM) is a plasmid-mediated enzyme that confers resistance to all current βlactam antibiotics, except for aztreonam. There are currently close to 40 different NDM types that may be found in a variety of gram-negative species, with NDM-1 recognized throughout the world. NDM is widely and rapidly disseminated throughout the *Enterobacterales*, as well as other gram-negative bacteria. The plasmids encoding NDM are easily transferable and capable of wide rearrangement, suggestive of extensive transmission, as well as plasticity, amongst bacterial populations.
OXA-48 like	An oxacillinase (OXA) β-lactamase that is part of a group of primarily plasmid-mediated enzymes that confer resistance to penicillins, cephalosporins, and carbapenems. The blaOXA-48 gene and its variants have been identified in various gram-negative bacteria in the *Enterobacterales*. OXA-48 hydrolyzes penicillins at a high level and carbapenems at a low level, with greater activity against imipenem than meropenem, and demonstrates extremely weak activity against expanded-spectrum cephalosporins.
vanA/B	Vancomycin resistance in *Enterococcus spp*. is conferred by the *vanA* and *vanB* genes. The prevalence of vancomycin-resistant enterococcus (VRE) has increased rapidly, with VRE accounting for 60% of E. faecium and 2% of *E. faecalis* isolated from the bloodstream. Both the *vanA* and *vanB* gene clusters are borne on mobile genetic elements (transposons) and can be located either on the chromosome or

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	carried on a plasmid. Enterococci carrying *vanA* or *vanB* are resistant to high levels of vancomycin.
VIM	Verona Integron-Encoded Metallo-β-Lactamase (VIM) is an integron-encoded carbapenemase. There are reports of both plasmid and chromosomal localization of the blaVIM integron, however, the majority of blaVIM alleles are found on plasmids. There are over 60 distinct VIM types. VIMs are found mainly in gram-negative bacteria, including *Enterobacterales*, with a vast majority associated with various species of *Pseudomonas spp.*

III. PRINCIPLE:

A. The BioFire® BCID2 Panel pouch is a closed system disposable that stores all the necessary reagents for sample preparation, polymerase chain reaction (PCR), and detection in order to isolate, amplify, and detect nucleic acid from multiple pathogens and antimicrobial resistance genes contained in blood culture samples identified as positive by a continuous monitoring blood culture system. After sample collection, the user injects hydration solution and sample combined with BioFire® FilmArray® Sample Buffer into the pouch, places the pouch into a BioFire® FilmArray® Instrument module, and starts a run.

B. During a run, the BioFire System:

1. Lyses the sample by agitation (bead beating) in addition to chemical lysis mediated by the Sample Buffer.
2. Extracts and purifies all nucleic acids from the sample using magnetic bead technology.
3. Performs nested multiplex PCR by:
    a. First performing a single, large volume, massively multiplexed reaction (PCR1)
    b. Then performing multiple single plex second-stage PCR reactions (PCR2) to amplify sequences within the PCR1 products
4. Uses endpoint melting curve data to detect and generate a result for each target on the BioFire® BCID2 Panel array.

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IV. SPECIMEN COLLECTION AND HANDLING:

A. **Specimen Collection**

1. Refer to the Laboratory Test Directory (LTD) for detailed collection information.
2. Collect and submit a blood culture set (one aerobic and one anaerobic blood culture bottle).

B. **Specimen**

1. Blood culture bottles identified as positive by a continuous monitoring blood culture system.

C. **Shipping and Handling**

1. Maintain positive blood culture bottles at room temperature (20-26°C or 68-78.8°F) and transport to the Microbiology Laboratory.
2. Samples should be processed and tested with the BioFire® BCID2 Panel as soon as possible.
3. Specimens not processed immediately can be kept up to 24 hours at room temperature or on the blood culture system after positivity.

D. **Rejection Criteria**

1. Specimens delayed in transit or testing after being flagged as positive on the blood culture system (>24 hours at room temperature or on the blood culture system).

E. **Storage**

1. Positive blood bottles will be held for one week at room temperature (20-26°C or 68-78.8°F)
2. Negative blood bottles will be discarded after testing is complete

V. REAGENTS:

A. BioFire® BCID2 Panel Kit (30-test kit, RFIT-ASY-0147)

1. Individually packaged BioFire® BCID2 Panel pouches
2. Single use (1mL) Sample Buffer ampoules
3. Single use pre-filled (1.5mL) BioFire® FilmArray® Hydration Injection Vials (blue cap)
4. Single use BioFire® FilmArray® Sample Injection Vials (red cap)
5. Individually packaged Transfer Pipettes

B. Other materials required

1. Blood Culture Bottles
2. Sub-Culture Airway Needles
3. Sterile secondary container
4. BioFire® FilmArray® Pouch Loading Station
5. 10% bleach (cleaning)
6. 70% ethanol (cleaning)

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VI. EQUIPMENT:

A. BioFire® 2.0 or BioFire® Torch Systems including one or more modules and accompanying software

B. Biological Safety Cabinet (BSC), class B type II

VII. QUALITY CONTROL (QC):

A. **Internal Controls**

1. Two process controls are included in each pouch:
   a. **DNA Process Control** - Assay targets a DNA 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, PCR1, dilution, PCR2, and DNA melting. A positive control result indicates that all steps carried out in the BioFire BCID2 Panel pouch were successful.
   b. **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.
2. 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.

B. **External Controls** (Maine Molecular Quality Controls, Inc., Saco, ME. FilmArray BCID2 Control Panel

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1. Positive and negative controls need to be ran every new lot, shipment, and or monthly which ever come first.
2. Controls are tested the same as patient samples, see Procedure section for protocol.
3. Results need to be recorded on the QC log sheet and reviewed by lab director or designee every 30 days.

VIII. PROCEDURE:

A. Positive blood culture bottles are Gram stained and sub-cultured according to the Blood Culture procedure.

B. Perform the BCID2 panel on the first positive blood culture bottle per inpatient admission.

   1. Due to limited clinical utility, the BCID2 panel will **not** be performed on positive blood cultures with only gram-positive bacilli identified in the Gram stain unless Listeria is suspected.

C. Subsequent BCID2 panels will only be performed if the Gram stain differs from the original result.

D. Order the appropriate BCID2 panel in the laboratory information system (LIS) based on the blood culture Gram stain result.

E. **Note**: Use clean gloves and other Personal Protective Equipment (PPE) when handling pouches and samples. Only prepare one BioFire BCID2 Panel at a time and change gloves between samples and pouches. Once sample is added to the pouch, promptly transfer to the instrument to start the run. After the run is complete, discard pouch in a biohazard container.

F. **Step 1: Prepare Pouch**

   1. Thoroughly clean the work area and the Pouch Loading Station with 10% bleach followed by 70% ethanol.
   2. Change gloves.
   3. Remove the pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective canister.
   4. Check the expiration date on the pouch. Do not use expired pouches.
   5. 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.
   6. Place a red-capped **Sample Injection Vial** into the red well of the Pouch Loading Station.
   7. Place a blue-capped **Hydration Injection Vial** into the blue well of the Pouch Loading Station.

G. **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.

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a. 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.

   a. Flip the bar code 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.

   b. 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.*

H. **Step 3: Prepare Sample Mix**

1. Add the Sample Buffer to the **Sample Injection Vial**.

   a. Hold the Sample Buffer ampoule with the tip facing up.

   b. Avoid touching the ampoule tip during handling, as this may introduce contamination.

   c. Firmly pinch at the textured plastic tab on the side of the ampoule until the seal snaps.

   d. Invert the ampoule over the red cap **Sample Injection Vial** an dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.

   e. Avoid squeezing the ampoule additional times to avoid foaming.

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 sub-culture Airway Needle remove blood (>0.2mL) into a sterile secondary container. Draw the blood culture sample from the secondary tube to the second line (0.2mL) of the transfer pipette and dispense into sample Buffer in **Sample Injection Vial**. Tightly close the lid of the Sample Injection Vial and discard transfer pipette.

   a. **Alternatively**: Use a syringe and withdraw 0.2mL of blood culture sample through the bottle septum, taking care to avoid the formation of bubbles. Add sample directly into Sample Buffer in **Sample Injection Vial**. Discard syringe and tightly close the lid of the **Sample Injection Vial**.

5. Remove the Sample Injection Vail from the Pouch Loading Station and invert the vial at least 3-10 times to mix.

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

I. **Step 4: Load Sample Mix**

1. Slowly twist to unscrew the **Sample Injection Vial** from the red cap and wait 5 seconds with the vial resting in the cap. This is to reduce the risk of dripping and contamination from the sample.

2. Lift the **Sample Injection Vial**, leaving the 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 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.

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a. Flip the bar code label down and check to see that fluid has entered the reagent well next to the sample loading port.

b. 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 **Hydration Injection Vial**.

6. Record the Sample ID in the area provided on the pouch label and remove the pouch from the Pouch Loading Station.

J. **Step 5: Run Pouch**

1. The BioFire® FilmArray® Software includes step-by-step, on-screen instructions that guide the operator through performing a run. Brief instructions for BioFire® Torch Systems are given below. Refer to the appropriate BioFire System Operator's Manual for more detailed instructions.

   a. Ensure that the BioFire Torch system is powered on

   b. Select an available module on the touch screen or scan the bar code on the pouch using the barcode scanner.

   c. Pouch identification (Lot Number and Serial Number), Pouch Type, and Procedure information will be automatically entered when the bar code is scanned. If it is not possible to scan the bar code, 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 bar code.

   d. Enter the Sample ID. The Sample ID can be entered manually or scanned in by using the bar code scanner when a bar coded Sample ID is used.

   e. Insert the pouch into the available module.

      i. Ensure that 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.

   f. 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.

   g. Enter a user name and password, then select Next.

   h. 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.

   i. At the end of the run, remove the partially ejected pouch, then immediately discard it in a biohazard waste container.

   j. 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. Interfaced results will cross automatically into LIS.

K. **Repeat Testing**

1. If the test fails or is invalid, repeat with a new pouch on the same module or a different one.

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IX. INTERPRETATION:

A. **Result Summary**

1. 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.

Result	Explanation	Action
Detected*	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) for the organism for (or ARM) 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 for (or ARM) were NEGATIVE	Report Results
Invalid	The run were not successful (Failed) OR The run did not complete successfully (Run status displayed as: Aborted, Incomplete, Instrument Error, or Software Error)	Note failure and repeat test
N/A	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) for the organism associated with the ARM) were NEGATIVE so the results of the AMR are not applicable to the test results.	Report Results
* If four or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.		

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B. **Organism and Antimicrobial Resistance Gene Interpretation**

1. 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.

C. **Result Interpretation for Gram-Positive Bacteria**

1. The BioFire® BCID2 Panel contains assays for the specific detection of the major species associated with Enterococcus spp. bloodstream infections (*Enterococcus faecium* and *Enterococcus faecalis*), *Listeria monocsytogene*, as well as clinically important *Staphylococcus spp.* (*S. aureus, S. epidermidis,* and *S. lugdunensis*) and *Streptococcus spp.* (*S. agalactiae, S. pneumoniae,* and *S. pyogenes*). Results for these gram-positive bacteria are reported as Detected or Not Detected based on an individual corresponding assay result. If the assay is positive the result will be Detected, and if the assay is negative, the result will be Not Detected.
   a. ***Staphylococcus spp.***
      i. The BioFire® BCID2 Panel contains four assays for the detection of *Staphylococcus species*. Species-specific genus-level are included for the detection of *Staphylococcus aureus, Staphylococcus epidermidis,* and *Staphylococcus lugdunensis*. The fourth assay is a genus-level assay (*Staphylococcus*) designed to react with Staphylococcus species not specifically identified by one of the other assays on the panel. The BioFire® Software integrates the results of all four assays into a *Staphylococcus spp.* result. If all four assays are negative, the test result will be *Staphylococcus spp.* Not Detected. Alternatively, if any of the four assays are positive, the test result will be *Staphylococcus spp.* Detected and results for each species-specific assay will also be reported independently.
   b. ***Streptococcus spp.***
      i. The BioFire® BCID2 Panel contains four assays for the detection of *Streptococcus* species. Species-specific assays are included for the detection of Group A Strep (Spyogenes), Group B Strep (Sagalactiae), and S. pneumoniae (Spneumoniae). The fourth assay is a genus-level assay (*Streptococcus*) designed to react with most Viridans group and other *Streptococcus* species that are not specifically identified by one of the other assays on the panel. The BioFire Software integrates the results of all four Streptococcus assays into a *Streptococcus spp.* Result. If all four assays are negative, the test result will be *Streptococcus spp.* Not Detected. Alternatively, if any of the four assays are positive, the test result will be *Streptococcus spp*. Detected and results for each species-specific assay will also be reported independently.

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D. **Results Interpretation for Gram-Negative Bacteria**

1. The BioFire® BCID2 Panel contains assays for the specific detection of many gram-negative species associated with bloodstream infections. Species are identified individually (*Bacteroides fragilis, Escherichia coli, Haemophilus influenzae, Klebsiella aerogenes, Klebsiella oxytoca, Neisseria meningitidis, Pseudomonas aeruginosa, Serratia marcescens,* and *Stenotrophomonas maltophilia*) or as complex, group, or genus results (*Acinetobacter calcoaceticus-baumannii complex, Enterobacter cloacae complex, Klebsiella pneumoniae group, Proteus spp.,* and *Salmonella spp.*). Each of these is reported as Detected or Not Detected based on an individual corresponding assay result. If the assay is positive, the result will be Detected; if the assay is negative, the result will be Not Detected.
   a. ***Enterobacterales***
      i. The BioFire® BCID2 Panel contains ten assays for the detection of most species within multiple families of the order *Enterobacterales*. Two assays (Enteric1 and Enteric2) are designed to react with relevant (and some non-relevant) species within the following families: *Enterobacteriaceae, Erwiniaceae, Hafniaceae, Morganellaceae, Yersiniaceae, Pectobacteriaceae,* and *Budviciaceae*; though species within the latter two families are generally not associated with human disease.

ii. The BioFire® BCID2 Panel also contains eight other assays for the detection of specific species, genera, or groups of species within the *Enterobacterales* order including *Enterobacter cloacae complex, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae group, Proteus spp., Salmonella spp.,* and *Serratia marcescens*.

iii. The BioFire® Software integrates the results of all ten assays into an *Enterobacterales* result. If all ten assays are negative, the test result will be *Enterobacterales* Not Detected. Alternatively, if any of the ten assays are positive, the test result will be *Enterobacterales* Detected and results for the genus, complex, group, or species-specific assays will also be reported independently.

E. **Results Interpretation for Antimicrobial Resistance (AMR) Genes**

1. The BioFire® BCID2 Panel contains assays for the specific detection of several genetic determinants of resistance to multiple classes of antibiotics found in select gram-positive (mecA/C, mecA/C, and MREJ, and vanA/B) or gram-negative bacteria (CTX-M, IMP, KPC, mcr-1, NDM, OXA-48, and VIM). Results for the AMR genes are not reported unless an applicable bacterium is also detected, therefore the results are based on multiple assays. The results for each of the antimicrobial resistance genes will be listed as:
   a. **Detected** – when an applicable bacterium is detected AND the antimicrobial resistance gene assay(s) are positive.
   b. **Not Detected** – when an applicable bacterium is detected AND the antimicrobial resistance gene assay(s) are negative.
   c. **N/A** – when all applicable bacteria are Not Detected, regardless of the result for the antimicrobial resistance gene assay(s).
2. Each AMR gene result is associated with a single corresponding AMR gene assay [except for the mecA/C and MREJ (MRSA) result] and one or more assay(s) for the detection of applicable bacteria.
3. The mecA/C result is intended to aid in the identification of methicillin-resistant *Staphylococcus epidermidis* and *Staphylococcus lugdunensis*. When the Sepidermidis and/or Slugdunensis assay(s) are positive, the mecA/C result will be reported as Detected or Not Detected based on whether the mecA/C assay is positive or negative, respectively. The Saureus and Staphylococcus assays are not considered in the reporting of the mecA/C result, except for the mecA/C and MREJ (MRSA) result, which is dependent on both the mecA/C assay and the MREJ assay. Detection of *Staphylococcus aureus* and positive mecA/C and MREJ results is indicative of methicillin-resistant *Staphylococcus aureus* (MRSA).

F. **Results Interpretation for Yeast**

1. Species-specific assays are included in the BioFire® BCID2 Panel for each of the five most common Candida species associated with BSI (*Candida albicans, Candida glabrata, Candida krusei, Candida parapsilosis,* and *Candida tropicalis*), the emerging pathogen *Candida auris*, and two species of *Cryptococcus (Cryptococcus neoformans/gattii)*. Results for all yeast are reported as Detected or Not Detected based on an individual corresponding assay result. If the assay is positive the result will be Detected, and if the assay is negative, the result will be Not Detected.

X. REPORTING:

A. **Result Reporting**

1. Before releasing results in LIS, review results for the run to verify correlation between the gram stain

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and the run report.

2. If the Gram stain matches the run report the following result interpretations are available for reporting in LIS:
   a. **NOT DETECTED**
      i. For each analyte with a Negative result, the report will read: "Not Detected"
   b. **DETECTED**
      i. For each analyte with a Positive result, report will read: "DETECTED: ABNORMAL RESULT"
      ii. Significant (abnormal) flag will trigger automatically
   c. **INVALID**
      i. Manually report all analytes as INVALID.
      ii. When testing and retesting of patient specimen results in 'No Result' test results, report will read:
   d. **Comments**
      i. Each order will also have a comment on report, noting the testing methodology: " Nucleic Acid Amplification (NAA)/Polymerase Chain Reaction (PCR).
3. If the Gram stain does not match the BCID2 run report, re-read the Gram stain. For example, gram positive cocci in clusters seen on Gram stain, but BCID2 run report identified *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
   a. If additional organism identified after Gram stain review, order additional BCID2 panel and release results.
   b. If additional organism not identified after Gram stain review, do not report the organism(s) that do not match and wait for culture confirmation.

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B. **Result Review – Download via Interface**
   1. All results that are acceptable as outlined in above Retesting, Results, and Reporting sections will be released to LIS for review.
   2. Only the results that qualify for retesting as stated in the "Retesting" section must be reviewed by the technologist prior to reporting.

C. **Result Review – Manual Entry.** Please note: Most common occurrence for manual entry will be in situations in which the interface is not functional. All manual results MUST be reviewed twice.
   1. The technologist performing the test must review the results entered in the LIS work card against the results printed on the instrument run log and the patient log sheet. Once verified, the technologist will save the result but will not status as final.
   2. A second review must than take place. The preferred method is to have a separate Medical Technologist review all test results, one by one, that were saved by the preceding technologist. If a second technologist is not present the resulting technologist must go through the same review process themselves. Once the review is complete and result accuracy has been verified, status as final and release the report to chart.
   3. The technologist performing the review MUST access all the following for accuracy:

a. Instrument logs

b. Result Review via LIS.

4. If any results are incorrectly transcribed to the patient logs from the instrument logs, or if incorrect results are entered into the LIS work card:

    a. Correct the mistake, initial the correction and then status as preliminary or final as needed and release the report to chart.

5. If an error is discovered after this review:

    a. Immediately notify a manager. If unavailable, email all information to them.

    b. Correct the report. All corrected results must be called to floor or physician accordingly.

XI. LIMITATIONS:

A. Results from this test must be correlated with clinical history, epidemiological data, and other data available to the clinician evaluating the patient.

B. This test has not been validated for testing samples other than human blood culture samples identified as positive by a continuous monitoring blood culture system.

C. Blood culture samples must be tested within 24 hours of being flagged as positive by a continuous monitoring blood culture system.

D. This product should not be used to test blood culture media that contain charcoal. Charcoal containing media may contain nonviable organisms and/or nucleic acid at levels that can be detected by the BioFire BCID2 Panel.

E. Any blood culture media may contain non-viable organisms and/or nucleic acid at levels that can be detected by the BioFire® BCID2 Panel leading to false positive test results. Typically, these false positives may present with more than one positive result because the BioFire® BCID2 Panel may also detect the organism that is growing in the culture bottle.

F. The BioFire® BCID2 Panel may not distinguish mixed cultures when two or more species of the same genus or organism group are present in a specimen (e.g., *Staphylococcus aureus* and *Staphylococcus hominis*).

G. In mixed cultures, the BioFire BCID2 Panel may not identify all targeted organisms in the specimen, depending upon the concentration of each target present. For example, false negative results for *Pseudomonas aeruginosa* or *Stenotrophomonas spp.* may occur if another organism is present in the blood culture. Conversely, standard subculture methods may also not identify all organisms in a mixed culture, depending upon the concentration and growth characteristics of each organism present.

H. Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for the antimicrobial resistance gene assays does not indicate antimicrobial susceptibility. Subculturing and standard susceptibility testing of isolates are required to determine antimicrobial susceptibility.

I. The results for the antimicrobial resistance gene assays do not specifically link the resistance gene to the applicable bacteria detected. In mixed cultures, the resistance gene may be associated with any of the applicable bacteria detected or an organism that was not detected by the panel.

J. The detection of bacterial, yeast, and antimicrobial resistance gene nucleic acid is dependent upon proper sample collection, handling, 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 or false

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negative values resulting from improperly collected, transported, or handled samples.

K. A negative BioFire BCID2 Panel result does not exclude the possibility of bloodstream infection. Negative test results may occur from sequence variants in the region targeted by the assay, the presence of inhibitors, technical error, sample mix-up, or an infection caused by an organism not detected by the panel. Test results may also be affected by concurrent antibacterial/antifungal therapy or levels of organism in the sample that are below the limit of detection for the test (especially in the case of mixed cultures). Negative results should not be used as the sole basis for diagnosis, treatment, or other management decisions.

L. There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay. False positives and false negatives can be the result of a variety of sources and causes. It is important that results be used in conjunction with other clinical, epidemiological, or laboratory information.

M. If four or more distinct organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.

N. BioFire® BCID2 Panel assays can cross-react with several organisms, typically closely related or near-neighbor species to those detected by the panel. All confirmed or predicted cross-reactive species have been identified, see package insert for full list of organisms.

O. The BioFire® BCID2 Panel Ctropicalis assay may cross-react with high titers of *C. parapsilosis* present in a sample and the Cparapsilosis assay may cross-react with high titers of C. tropicalis. Detected results for both C. tropicalis and C. parapsilosis in the same sample may be due to cross-reactivity or may be due to both organisms being present in the blood culture.

P. Borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) and moderately resistant *S. aureus* (MODSA) strains demonstrate reduced susceptibility to oxacillin due to hyperproduction of β-lactamases or modification of penicillin-binding proteins respectively. BORSA and MODSA strains do not contain the mecA or mecC gene. A mecA/C and MREJ (MRSA) Not Detected result will be reported by the BioFire BCID2 Panel for these strains.

Q. The vanA/B result is not reported in the absence of *Enterococcus faecalis* or *Enterococcus faecium* detection and will therefore not be reported for blood cultures containing other vancomycin-resistant Enterococci or vancomycin-resistant *Staphylococcus aureus* (VRSA).

R. Continuous monitoring blood culture systems may falsely signal positive in a low percentage of bottles (estimated to be near 1%) when no organisms are growing in the sample. This may be due to hyperleukocytosis (very high white blood cell counts).

S. The performance of the BioFire BCID2 Panel has not been established for monitoring the treatment of infection with any of the panel organisms.

XII. SPECIAL NOTES:

A. **Preventing organism contamination**
Due to the sensitive nature of the BioFire® BCID2 Panel, it is important to guard against contamination of the sample and work area by carefully following the testing process outlined in this instruction document, including these guidelines:

1. Positive blood culture samples contain high concentrations of organisms; careful adherence to the sample processing steps is important. Samples should be processed in a clean biosafety cabinet if available.

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2. It is recommended to avoid handling specimens or pouches in an area used to routinely process primary samples (excluding positive blood culture bottles), for bacterial or fungal Gram stains, rapid antigen testing, and/or cultures, unless the area is thoroughly cleaned first.
3. Prior to processing specimens, thoroughly clean both the work area and the Pouch Loading Station with freshly prepared 10% bleach or a similar disinfectant. To avoid residue build-up and potential damage to the specimen or interference from disinfectants, wipe disinfected surfaces with ethanol.
4. Specimens and pouches should be handled and/or tested one at a time. Always change gloves and clean the work area between each pouch and specimen.
5. Use clean gloves when removing Sample Buffer ampoules and Sample/Hydration Injection Vials from bulk packaging bags and reseal bulk packaging bags when not in use.

B. **Preventing amplicon contamination**

1. A common concern with PCR-based assays is false positive results caused by contamination of the work area with PCR amplicon. Because the BioFire BCID2 Panel pouch is a closed system, the risk of amplicon contamination is low, provided that the pouches remain intact after the test is completed. Adhere to the following guidelines, in addition to those above, to prevent amplicon contamination:
   a. Discard used pouches in a biohazard container immediately after the run has completed.
   b. Avoid excessive handling of pouches after test runs.
   c. Change gloves after handling a used pouch.
   d. Avoid exposing pouches to sharp edges or anything that might cause a puncture.

C. **Monitoring for contamination**

1. An investigation will be conducted on physician complaints of false positive results or an unexpected increase in positive or unusual results. Testing data will be reviewed during an investigation to determine if corrective action is needed.

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XIII. REFERENCES:

1. BioFire Blood Culture Identification 2 (BCID2) Panel, package insert, RFIT-PRT-0841-02 June 2020

Attachments

RFIT-PRT-0370 FilmArray BCID (FAIV) Quick Guide

Approval Signatures

Step Description	Approver	Date
	Jeremy Powers: Chief, Pathology	3/1/2022
	Ann Marie Blenc: System Med Dir, Hematopath	2/28/2022
Policy and Forms Steering Committee Approval (if needed)	Corey Webber: Mgr Laboratory	2/28/2022
Policy and Forms Steering Committee	Gail Juleff: Project Mgr Policy	2/28/2022

Step Description	Approver	Date
Approval (if needed)		
	Joyce Mitchell: Mgr Laboratory	2/28/2022
	Corey Webber: Mgr Laboratory	2/18/2022
	Daniel Ortiz: Technical Dir, Microbiology	2/18/2022
	Corey Webber: Mgr Laboratory	2/18/2022

Applicability

Dearborn, Royal Oak

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