### BioFire® Blood Culture Identification 2 (BCID2) Panel Testing

### Purpose

This procedure provides instructions for testing positive blood culture samples using the  
BioFireBCID2 Panel Kit.

### Background

The BioFire BCID2 Panel is a multiplexed nucleic acid test intended for use with BioFire® FilmArray® 2.0 or 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 a continuous monitoring blood culture system. Results are intended to be interpreted in conjunction with Gram stain results. The following organism types and subtypes are identified using the BioFire BCID2 Panel:

**Gram-Positive Bacteria**

* *Enterococcus faecalis*
* *Enterococcus faecium*
* *Listeria monocytogenes*
* *Staphylococcus* spp.
  + *Staphylococcus aureus*
  + *Staphylococcus epidermidis*
  + *Staphylococcus lugdunensis*
* *Streptococcus* spp.
  + *Streptococcus agalactiae* (Group B)
  + *Streptococcus pneumonia*
  + *Streptococcus pyogenes* (Group A)

**Gram-Negative Bacteria**

* *Acinetobacter calcoaceticus-baumannii* complex
* *Bacteroides fragilis*
* *Haemophilus influenzae*
* *Neisseria menigitidis* (encapsulated)
* *Psuedomonas aeruginosa*
* *Stenotrophomonas maltophilia*
* *Enterobacterales*
  + *Enterobacter cloacae* complex
  + *Escherichia coli*
  + *Klebsiella aerogenes*
  + *Klebsiella oxytoca*
  + *Klebsiella pneumonia* 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**

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

The BioFire BCID2 Panel contains assays for the detection of genetic determinants associated with resistance to methicillin (*mecA/C* and *mecA/C* in conjunction with MREJ), vancomycin (*vanA* and *vanB*), ß-lactams including penicillins, cephalosporins, monobactams, and carbapenems (*bla*CTX-M, *bla*IMP, *bla*KPC, *bla*NDM, *bla*OXA48-like, *bla*VIM) to aid in the identification of potentially antimicrobial-resistant organisms in positive blood culture samples. In addition, the panel includes an assay for the detection of the mobilized genetic determinant *mcr-1*, an emerging marker of public health importance. The antimicrobial resistance gene or marker detected may or may not be associated with the agent responsible for disease. Negative results for these select antimicrobial resistance gene and marker assays do not indicate susceptibility, as multiple mechanisms of resistance to methicillin, vancomycin, ß-lactams, and colistin exist.

### Principle of the Procedure

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 Sample Buffer into the pouch, places the pouch into a BioFire® FilmArray® Instrument module, and starts a run. The entire run process takes about an hour. Additional detail can be found in the appropriate BioFire® FilmArray® System Operator’s Manual.

During a run, the BioFire System:

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

### Specimen

|  |  |
| --- | --- |
| **Specimen Type** | **Blood Culture Samples** identified as positive by a continuous monitoring blood culture system. |
| **Minimum Sample Volume** | 0.2 mL (200 µL) |
| **Sample Age** | Specimens should be processed and tested with the BioFire BCID2 Panel as soon as possible after positive bottle indication by a blood culture system.  If not processed immediately, specimens can be kept:   * At room temperature for up to 24 hours (15-25 °C) after positivity * In the blood culture system for up to 24 hours after positivity |

### Materials

|  |  |
| --- | --- |
| **Materials Provided** | **Materials Required But Not Provided** |
| Each kit contains sufficient reagents to test 30 samples (30-test kit; RFIT-ASY-0147):   * Individually packaged BioFire BCID2 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 System including:   + BioFire® 2.0 or BioFire® Torch systems including one or more modules and accompanying software   + Pouch Loading Station * Syringe capable of measuring 0.2 mL (200 µL) sample volume; OR alternate subculture device (e.g.needle-less safe subculture device) and sterile secondary container * 10% bleach solution or a similar disinfectant |

### Quality Control

**Process Controls**

Two process controls are included in each pouch:

**1. DNA Process Control**

The 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, reverse transcription, PCR1, dilution, PCR2, and DNA melting. A positive control result indicates that all steps carried out in the BioFire BCID2 Panel pouch were successful.

**2. PCR2 Control**

The PCR2 Control assay detects a DNA target that is dried into wells of the array along with the corresponding primers. A positive result indicates that PCR2 was successful

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.

**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 of an acceptable range (77.4-81.4°C for the DNA Process Control and 73.8-77.8°C 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.116,117 Refer to the appropriate BioFire® FilmArray® Operator’s Manual for instructions on obtaining control assay Tm values. The PCR2 Control is used in several BioFire® FilmArray® Pouch types (e.g., RP, BCID, GI, ME, and RP2) and can therefore be used to monitor the system when multiple pouch types are used on the same BioFire® System or Instrument

## External Controls

External controls should be used in accordance with laboratory protocols and the appropriate accrediting organization requirements, as applicable. Un-inoculated blood culture media can be used as an external negative control; however, external negative controls may not identify sporadic nucleic acid contamination in blood culture bottles. Previously characterized positive blood culture samples or samples spiked with well-characterized organisms can be used as external positive controls. Commercially produced control materials may also be available from other manufacturers; use according to the control manufacturer’s instructions.

### Procedure

Use clean gloves and other Personal Protective Equipment (PPE) when handling pouches and samples. Only prepare one BioFire BCID2 Panel pouch 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 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, 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.

**Alternatively:** Draw the desired amount of blood culture sample (> 0.2 mL) from the bottle into the syringe and transfer to a sterile secondary container OR remove blood culture sample (> 0.2 mL) using an alternate subculture device (*e.g.* needle-less safe subculture device) into a sterile secondary container. Draw the blood culture sample from the secondary container to the second line of the Transfer Pipette (0.2 mL) and add the sample to Sample Buffer in the Sample Injection Vial. Tightly close the lid of the Sample Injection Vial and discard the transfer pipette in a biohazard waste container.

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

### BioFire 2.0

1. Ensure that the BioFire 2.0 System (module[s] and computer) is powered on and the software is launched.
2. Follow on-screen instructions and procedures described in the Operator’s Manual to place the pouch in a module, enter pouch, sample, and operator information.
3. Pouch identification (Lot Number and Serial Number), Pouch Type, and Protocol 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. ***When selecting a Pouch Type manually, ensure that the Pouch Type matches the label on the BioFire BCID2 Panel pouch.***
4. 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.
5. 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.
6. Enter a user name and password in the Name and Password fields.
7. Review the entered run information on the screen. If correct, select Start Run.
8. 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.
9. When the run is finished, follow the on-screen instructions to remove the pouch, then immediately discard it in a biohazard waste container.
10. 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.

### BioFire® Torch

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. 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. 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 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.

### Interpretation of Results

## Assay Interpretation

When PCR2 is complete, the BioFire® Instrument performs a high-resolution DNA melting analysis on the PCR products and measures the fluorescence signal generated in each well (for more information see appropriate BioFire® System Operator’s Manual). The BioFire® Software then performs several analyses and assigns a final assay result. The steps in the analyses are described below.

**Analysis of melt curve.** The BioFire Software evaluates the DNA melt curve for each well of the PCR2 array to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature (Tm) of the curve and compares it against the expected Tm range for the assay. If the software determines that the Tm falls inside the assay-specific Tm range, the melt curve is called positive. If the software determines that the melt curve is not in the appropriate Tm range, the melt curve is called negative.

**Analysis of replicates.** Once positive melt curves have been identified, the software evaluates the replicates for each assay to determine the assay result. For an assay to be called positive, two associated melt curves must be called positive, and both Tms must be similar. Assays that do not meet these criteria are called negative.

## Organism and Antimicrobial Resistance Gene Interpretation

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 as shown in Table 2.

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, as discussed below.

***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.**

#### Table 2. 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* complex | | | |  | *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 krusei* | *Cryptococcus neoformans/gattii* | | | |
| *Candida auris* | | | | *Candida parapsilosis* |  | | | |
| *Candida glabrata* | | | | *Candida tropicalis* |  | | | |
| **Antimicrobial Resistance Genes** | | | | | | | | |
| CTX-M | KPC | | | *mecA/C* | NDM | | | *vanA/B* |
| IMP | *mcr-1a* | | | *mecA/C* and MREJ (MRSA) | OXA-48-like | | | VIM |

aAs of February 2020, the United States Food and Drug Administration has not established or recognized minimum inhibitory concentration (MIC) breakpoints for colistin antimicrobial susceptibility testing (AST) related to *mcr-1.*

### Results Interpretation for Gram-Positive Bacteria

The BioFire BCID2 Panel contains assays for the specific detection of the major species associated with *Enterococcus* bloodstream infections (*Enterococcus faecium* and *Enterococcus faecalis*) and *Listeria monocytogenes*, as well as clinically important Staphylococci (*S. aureus*, *S. epidermidis,* and *S. lugdunensis*) and Streptococci (*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.

Information about detection of specific subspecies, strains, isolates, or serotypes of gram-positive bacteria is provided in the Analytical Reactivity (Inclusivity) section (Table 87 – Table 97). Based on *in silico* analysis and empirical testing, each of the gram-positive species-specific assays is specific for detection of the indicated species with the exception of the Saureus assay, which will also amplify the closely related species of the *S. aureus*-complex (*S. argenteus* and *S. schweitzeri*), as noted in the Analytical Specificity (Cross-Reactivity and Exclusivity) section (Table 130).

In addition, the panel detects other species identified as *Staphylococcus* spp. and *Streptococcus* spp. based on the results of multiple assays, as described below.

###### ***Staphylococcus* spp.**

The BioFire BCID2 Panel contains four assays for the detection of *Staphylococcus* species. Species-specific assays 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 (see Table 90). The BioFire® Software integrates the results of all four assays into a *Staphylococcus* spp. result as shown in the Table 3. 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.

Based on testing and sequence analysis, it is predicted that five species within the *Staphylococcus* genus (*S. equorum*, *S. fluerettii*, *S. lentus*, *S. muscae,* and *S. rostri*) may not be detected by the panel, even at positive blood culture levels, due to sequence variation under the assay primers. Of these, only *S. equorum* has been reported to be isolated in a clinical setting. Sequence analysis predicts a low risk of cross-reactivity between the *Staphylococcus* assay and homologous sequences from *Aerococcus viridans*, *Enterococcus cecorum*, and *Granulicatella adiacens*, although only at very high concentrations.

#### Table 3. Assay and Results Interpretation for the *Staphylococcus* spp., *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus lugdunensis* Test Results

| **BioFire BCID2 Panel Results** | ***Staphylococcus* assay** | **Saureus assay** | **Sepidermidis assay** | **Slugdunensis assay** | **Description** |
| --- | --- | --- | --- | --- | --- |
| *Staphylococcus* spp. Not Detected  *Staphylococcus aureus* Not Detected  *Staphylococcus epidermidis* Not Detected  *Staphylococcus lugdunensis* Not Detected | Negative | Negative | Negative | Negative | No *Staphylococcus* species detected in the sample |
| ***Staphylococcus* spp. Detected**  *Staphylococcus aureus* Not Detected  *Staphylococcus epidermidis* Not Detected  *Staphylococcus lugdunensis* Not Detected | **Positive** | Negative | Negative | Negative | One or more *Staphylococcus* species detected in the sample  (not *S. aureus*, *S. epidermidis*, or *S. lugdunensis*) |
| ***Staphylococcus* spp. Detected**  ***Staphylococcus aureus*****Detected**  *Staphylococcus epidermidis* Not Detected  *Staphylococcus lugdunensis* Not Detected | Any result | **Positive** | Negative | Negative | *Staphylococcus aureus* detected in the sample  Note: additional *Staphylococcus* species (not *S. epidermidis* or *S. lugdunensis*) may also be in the sample |
| ***Staphylococcus* spp. Detected**  *Staphylococcus aureus* Not Detected  ***Staphylococcus epidermidis*****Detected**  *Staphylococcus lugdunensis* Not Detected | Any result | Negative | **Positive** | Negative | *Staphylococcus epidermidis* detected in the sample  Note: additional *Staphylococcus* species (not *S. aureus* or *S. lugdunensis*) may also be in the sample |
| ***Staphylococcus* spp. Detected**  *Staphylococcus aureus* Not Detected  *Staphylococcus epidermidis* Not Detected  ***Staphylococcus lugdunensis*****Detected** | Any result | Negative | Negative | **Positive** | *Staphylococcus lugdunensis* detected in the sample  Note: additional *Staphylococcus* species (not *S. aureus* or *S. epidermidis*) may also be in the sample |

**NOTE: Multiple Staphylococcus species assays may be positive in a single sample. If this occurs, the test result for each species with a positive assay will be reported as Detected.**

###### ***Streptococcus* spp**.

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 as shown in Table 4. 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.

Based on testing and analysis of available sequence data, all species within the *Streptococcus* genus will be amplified by one or more of the assays on the panel at positive blood culture levels. However, there are some species (*Streptococcus equi*, *S. entericus*, *S. halitosis*, *S.hyovaginalis*, *S. minor,* and *S. pantholopis*) and variant sequences identified as *S. minor*, *S. oralis*, *S. sobrinus*, *S. suis,* and *S. uberis* that may be amplified less efficiently than others and may not be detected if present in a blood culture at a concentration lower than approximately 7.6E+06 CFU/mL.

#### Table 4. Assay and Results Interpretation for the *Streptococcus* spp., *Streptococcus agalactiae* (Group B), *Streptococcus pneumoniae*, and *Streptococcus pyogenes* (Group A) Test Results

| **BioFire BCID2 Panel Results** | **Streptococcus Assay** | **Sagalactiae Assay** | **Spneumoniae Assay** | **Spyogenes Assay** | **Description** |
| --- | --- | --- | --- | --- | --- |
| *Streptococcus* spp. Not Detected  *Streptococcus agalactiae* (Group B) Not Detected  *Streptococcus pneumoniae* Not Detected  *Streptococcus pyogenes* (Group A) Not Detected | Negative | Negative | Negative | Negative | No *Streptococcus* species detected in the sample |
| ***Streptococcus* spp. Detected**  *Streptococcus agalactiae* (Group B) Not Detected  *Streptococcus pneumoniae* Not Detected  *Streptococcus pyogenes* (Group A) Not Detected | **Positive** | Negative | Negative | Negative | One or more *Streptococcus* species detected in the sample  (not *S. agalactiae*, *S. pneumoniae*, or *S. pyogenes*) |
| ***Streptococcus* Detected**  ***Streptococcus agalactiae* (Group B)** **Detected**  *Streptococcus pneumoniae* Not Detected  *Streptococcus pyogenes* (Group A) Not Detected | Any result | **Positive** | Negative | Negative | *Streptococcus agalactiae* detected in the sample.  Note: additional *Streptococcus* species (not *S. pneumoniae* or *S. pyogenes*) may also be in the sample |
| ***Streptococcus* Detected**  *Streptococcus agalactiae* (Group B) Not Detected  ***Streptococcus pneumoniae* Detected**  *Streptococcus pyogenes* (Group A) Not Detected | Any result | Negative | **Positive** | Negative | *Streptococcus pneumoniae* detected in the sample  Note: additional *Streptococcus* species (not *S. agalactiae* or *S. pyogenes*) may also be in the sample |
| ***Streptococcus* Detected**  *Streptococcus agalactiae* (Group B) Not Detected  *Streptococcus pneumoniae* Not Detected  ***Streptococcus pyogenes* (Group A)** **Detected** | Any result | Negative | Negative | **Positive** | *Streptococcus pyogenes* detected in the sample  Note: additional *Streptococcus* species (not *S. agalactiae* or *S. pneumoniae*) may also be in the sample |

**NOTE: Multiple Streptococcus species assays may be positive in a single sample. If this occurs, the test result for each species with a positive assay will be reported as Detected.**

### Results Interpretation for Gram-Negative Bacteria

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, and if the assay is negative, the result will be Not Detected.

In addition, the panel detects a large number of additional gram-negative species identified as *Enterobacterales* based on the results of multiple assays, as described below.

Information about the detection of specific subspecies, strains, isolates, or serotypes of gram-negative bacteria is provided in the Analytical Reactivity (Inclusivity) section (Table 98 – Table 112). Based on testing and *in silico* analysis, each of the gram-negative assays is specific for detection of the indicated genus, complex, group, or species with the exception of the cross-reactivities noted below and in the Analytical Specificity (Cross-Reactivity and Exclusivity) section (Table 130 and Table 131).

* *Bacteroides xylanisolvens*, a commensal species that naturally resides in the human intestine, can be misidentified as *Bacteroides fragilis* due to non-specific interaction with the Bfragilis assay. At particularly high concentration a cross-reactive interaction may also be seen with *Bacteroides caccae*, a species of the *B. fragilis* group.
* The *Enterobacter cloacae* complex is comprised of multiple species (*E. asburiae*, *E. cloacae*, *E. hormaechei*, *E. kobei*, *E. ludwigii, and E. mori*) that may all be identified as *E. cloacae* by phenotypic laboratory methods. The Ecloacae assay will detect each of these species and subspecies (e.g. *Enterobacter hormaechei* ssp. *xiangfangensis*) and will also cross-react with another closely related and more recently identified species, *Enterobacter bugandensis*. In addition, at high concentration, the Ecloacae assay will cross-react with *Trabulsiella guamensis* due to non-specific interaction of the assay with a distantly related gene sequence in this species.
* The Ecoli assay cross-reacts with *Shigella* species (*S. boydii*, *S. dysenteriae*, *S. flexneri*, and *S. sonnei*); which are practically indistinguishable from *E. coli* by both phenotypic and genetic analyses but are only very rarely isolated from blood culture. Cross-reactivity has also been observed with *Escherichia fergusonii*, a rare but emerging veterinary and human pathogen, and *Escherichia albertii* (only at high concentration), a species more typically associated with gastrointestinal infections.
* *Haemophilus aegyptius*, generally considered a subgroup or biotype of *Haemophilus influenzae*, is difficult to differentiate by most laboratory methods and will be detected as *Haemophilus influenzae* by the BioFire BCID2 Panel due to cross-reactivity.
* The Koxytoca assay will cross-react with two recently identified *Klebsiella* species, *K. grimontii* (identified in 2018; previously *Klebsiella oxytoca* phylogroup Ko6) and *K. michiganensis* (identified in 2013). The assay does not cross-react with other *Klebsiella* species; however, *K. pneumoniae* or *Raoultella ornithinolytica* can be misidentified as *K. oxytoca* by standard laboratory methods leading to instances of apparent false negative *K. oxytoca* results.
* The Salmonella assay will react with both species of *Salmonella* (*S. bongori* and *S. enterica*), including all known subspecies and serotypes. Although not detected when tested at a high concentration of ~7.0E+09 CFU/mL, sequence analysis predicts a low risk of cross-reactivity between this assay and a homologous gene sequence found in *Plesiomonas shigelloides*.
* The Proteus assay can cross-react with an insect-associated species (*Cosenzaea myxofaciens*) that was formerly classified as *Proteus myxofaciens*.

###### ***Enterobacterales***

The BioFire BCID2 Panel contains 10 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 twofamilies aregenerally not associated with human disease28.

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*.

The BioFire® Software integrates the results of all 10 assays into an *Enterobacterales* result as shown with examples in Table 5. If all 10 assays are negative, the test result will be *Enterobacterales* Not Detected. Alternatively, if any of the 10 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.

Based on testing and analysis of available sequence data, the Enteric1 and Enteric2 assays will amplify all *Enterobacteriaceae* evaluated, as well as most species of the other families within the *Enterobacterales* order. However, a few species of *Morganellaceae* (*Providencia heimbachae*, *Photorhabdus asymbiotica*, and *Arsenophonus nasoniae*) which are rarely or never isolated from human clinical samples, will not be detected. In addition, *Yersinia pseudotuberculosis* and *Mixta* (formerly *Pantoea*) *calida* and species with similar sequences may be amplified less efficiently than others and may not be detected if present in a blood culture at a concentration lower than approximately 1.1E+07 CFU/mL.

#### Table 5. Assay and Results Interpretation for the *Enterobacterales* Test Result

| **BioFire BCID2 Panel Results** | **Enteric1 and/or  Enteric2 assay** | **Ecloacae assay** | **Ecoli assay** | **Kaerogenes assay** | **Koxytoca assay** | **Kpneumoniae assay** | **Proteus assay** | **Salmonella assay** | **Smarcescens assay** | **Description** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Enterobacterales* Not Detected  *Enterobacter cloacae* complexNot Detected  *Escherichia coli* Not Detected  *Klebsiella aerogenes* Not Detected  *Klebsiella oxytoca* Not Detected  *Klebsiella pneumoniae* groupNot Detected  *Proteus* spp.Not Detected  *Salmonella* spp*.* Not Detected  *Serratia marcescens* Not Detected | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | No *Enterobacterales* detected in the sample. |
| ***Enterobacterales* Detected**  *Enterobacter cloacae* complexNot Detected  *Escherichia coli* Not Detected  *Klebsiella aerogenes* Not Detected  *Klebsiella oxytoca* Not Detected  *Klebsiella pneumoniae* Not Detected  *Proteus* spp.Not Detected  *Salmonella* spp*.* Not Detected  *Serratia marcescens* Not Detected | **Pos** | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | One or more *Enterobacterales* detected in the sample  (not *E. cloacae* complex, *E. coli*, *K. aerogenes*, *K. oxytoca*, *K. pneumoniae* group, *Proteus* spp., *Salmonella* spp., or *S. marcescens*) |
| ***Enterobacterales* Detected**  ***Enterobacter cloacae* complexDetected**  *Escherichia coli* Not Detected  *Klebsiella aerogenes* Not Detected  *Klebsiella oxytoca* Not Detected  *Klebsiella pneumoniae* groupNot Detected  *Proteus* spp.Not Detected  *Salmonella* spp*.* Not Detected  *Serratia marcescens* Not Detected | Any result | **Pos** | Neg | Neg | Neg | Neg | Neg | Neg | Neg | One or more species of the *Enterobacter cloacae* complex detected in the sample  Note: additional *Enterobacterales* may also be in the sample |
| ***Enterobacterales* Detected**  *Enterobacter cloacae* complexNot Detected  ***Escherichia coli* Detected**  *Klebsiella aerogenes* Not Detected  *Klebsiella oxytoca* Not Detected  *Klebsiella pneumoniae* groupNot Detected  *Proteus* spp.Not Detected  *Salmonella* spp*.* Not Detected  *Serratia marcescens* Not Detected | Any result | Neg | **Pos** | Neg | Neg | Neg | Neg | Neg | Neg | *Escherichia coli* detected in the sample  Note: additional *Enterobacterales* may also be in the sample |
| ***Enterobacterales* Detected**  *Enterobacter cloacae* complexNot Detected  *Escherichia coli* Not Detected  ***Klebsiella aerogenes* Detected**  *Klebsiella oxytoca* Not Detected  *Klebsiella pneumoniae* groupNot Detected  *Proteus* spp.Not Detected  *Salmonella* spp*.* Not Detected  *Serratia marcescens* Not Detected | Any result | Neg | Neg | **Pos** | Neg | Neg | Neg | Neg | Neg | *Klebsiella aerogenes* detected in the sample  Note: additional *Enterobacterales* may also be in the sample |
| ***Enterobacterales* Detected**  *Enterobacter cloacae* complexNot Detected  *Escherichia coli* Not Detected  *Klebsiella aerogenes* Not Detected  ***Klebsiella oxytoca* Detected**  *Klebsiella pneumoniae* groupNot Detected  *Proteus spp.* Not Detected  *Salmonella* spp*.* Not Detected  *Serratia marcescens* Not Detected | Any result | Neg | Neg | Neg | **Pos** | Neg | Neg | Neg | Neg | *Klebsiella oxytoca* detected in the sample  Note: additional *Enterobacterales* may also be in the sample |
| ***Enterobacterales* Detected**  *Enterobacter cloacae* complexNot Detected  *Escherichia coli* Not Detected  *Klebsiella aerogenes* Not Detected  *Klebsiella oxytoca* Not Detected  ***Klebsiella pneumoniae* groupDetected**  *Proteus* spp.Not Detected  *Salmonella* spp*.* Not Detected  *Serratia marcescens* Not Detected | Any result | Neg | Neg | Neg | Neg | **Pos** | Neg | Neg | Neg | One or more species in the *Klebsiella pneumoniae* group detected in the sample  Note: additional *Enterobacterales* may also be in the sample |
| ***Enterobacterales* Detected**  *Enterobacter cloacae* complexNot Detected  *Escherichia coli* Not Detected  *Klebsiella aerogenes* Not Detected  *Klebsiella oxytoca* Not Detected  *Klebsiella pneumoniae* groupNot Detected  ***Proteus* spp. Detected**  *Salmonella* spp*.* Not Detected  *Serratia marcescens* Not Detected | Any result | Neg | Neg | Neg | Neg | Neg | **Pos** | Neg | Neg | One or more *Proteus* species detected in the sample  Note: additional *Enterobacterales* may also be in the sample |
| ***Enterobacterales* Detected**  *Enterobacter cloacae* complexNot Detected  *Escherichia coli* Not Detected  *Klebsiella aerogenes* Not Detected  *Klebsiella oxytoca* Not Detected  *Klebsiella pneumoniae* groupNot Detected  *Proteus* spp. Not Detected  ***Salmonella* spp*.* Detected**  *Serratia marcescens* Not Detected | Any result | Neg | Neg | Neg | Neg | Neg | Neg | **Pos** | Neg | One or more *Salmonella* species detected in the sample  Note: additional *Enterobacterales* may also be in the sample |
| ***Enterobacterales* Detected**  *Enterobacter cloacae* complexNot Detected  *Escherichia coli* Not Detected  *Klebsiella aerogenes* Not Detected  *Klebsiella oxytoca* Not Detected  *Klebsiella pneumoniae* group Not Detected  *Proteus* spp. Not Detected  *Salmonella* spp*.* Not Detected  ***Serratia marcescens* Detected** | Any result | Neg | Neg | Neg | Neg | Neg | Neg | Neg | **Pos** | *Serratia marcescens* detected in the sample  Note: additional *Enterobacterales* may also be in the sample |

**NOTE: Multiple Enterobacterales assays may be positive in a single sample. If this occurs, the test result for each species with a positive assay will be reported as Detected.**

### 

### Results Interpretation for Antimicrobial Resistance (AMR) Genes

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, as described below.

The results for each of the antimicrobial resistance genes will be listed as:

* Detected – when an applicable bacterium is detected AND the antimicrobial resistance gene assay(s) are positive.
* Not Detected – when an applicable bacterium is detected AND the antimicrobial resistance gene assay(s) are negative.
* N/A – when all applicable bacteria are Not Detected, regardless of the result for the antimicrobial resistance gene assay(s).

Each AMR gene result is associated with a single corresponding AMR gene assay [with the exception of the *mecA/C* and MREJ (MRSA) result] and one or more assay(s) for the detection of applicable bacteria, as indicated in Table 6. Table 7 and Table 8 provide an example for how to interpret results when a corresponding AMR gene is detected with an associated organism. Table 7 describes how to interpret a *vanA/B* AMR gene result with corresponding gram-positive bacteria, while Table 8 describes how to interpret the KPC AMR gene result with corresponding gram-negative bacteria. All other AMR genes [with the exception of the *mecA/C* and MREJ (MRSA) result] follow these interpretation rules.

Information about detection of specific AMR gene typesis is provided in the Analytical Reactivity (Inclusivity) section (Table 113 – Table 122). Overall, each AMR gene assay was found to detect the majority of AMR gene types based on testing and analysis of available sequence data. However, some types and variant sequences identified may be amplified less efficiently or may not be detected (MREJ types xv, xviii, and xix; CTX-M-151; IMP-31, IMP-35, IMP-46, and IMP-63; OXA-54, OXA-416, and OXA-48-like types that lack carbapenemase activity; VIM-39, VIM-45, VIM-46, VIM-65, and VIM-67).

Information on cross-reactivity of AMR gene assays with related AMR genes or due to non-specific interactions are described in the Analytical Specificity (Cross-Reactivity and Exclusivity) section. Most AMR gene assays are specific for detection of the indicated AMR genes; however, cross-reactivity may be observed between AMR gene assays and related AMR genes (CTX-M with related *bla* genes and *vanA/B* with *vanM*) or due to non-specific interactions that are unlikely to be reported at blood culture titers (KPC with *Acinetobacter nosocomialis* and *Moraxella osloensis*) and/or in the absence of an applicable bacterium (CTX-M with *Acinetobacter schindleri*).

#### Table 6. Antimicrobial Resistance (AMR) Genes and Applicable Bacteria

| **BioFire BCID2 Panel**  **AMR Gene Result** | ***Enterococcus faecalis*** | ***Enterococcus faecium*** | ***Staphylococcus aureus*** | ***Staphylococcus epidermidis*** | ***Staphylococcus lugdunensis*** | ***Acinetobacter calcoaceticus-baumanii* complex** | ***Enterobacterales*** | ***Enterobacter cloacae* complex** | ***Escherichia coli*** | ***Klebsiella aerogenes*** | ***Klebsiella oxytoca*** | ***Klebsiella pneumoniae* group** | ***Proteus* spp.** | ***Salmonella* spp.** | ***Serratia marcescens*** | ***Pseudomonas aeruginosa*** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ***vanA/B*** | × | × |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| ***mecA/C*** |  |  |  | × | × |  |  |  |  |  |  |  |  |  |  |  |
| ***mecA/C* and MREJ (MRSA)** |  |  | × |  |  |  |  |  |  |  |  |  |  |  |  |  |
| ***mcr-1*** |  |  |  |  |  |  |  | × | × | × | × | × |  | × |  |  |
| **CTX-M** |  |  |  |  |  | × | × | × | × | × | × | × | × | × | × | × |
| **IMP** |  |  |  |  |  | × | × | × | × | × | × | × | × | × | × | × |
| **KPC** |  |  |  |  |  | × | × | × | × | × | × | × | × | × | × | × |
| **NDM** |  |  |  |  |  | × | × | × | × | × | × | × | × | × | × | × |
| **OXA-48-like** |  |  |  |  |  |  | × | × | × | × | × | × | × | × | × |  |
| **VIM** |  |  |  |  |  | × | × | × | × | × | × | × | × | × | × | × |

**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.***

#### Table 7 . Possible Assay and Corresponding *vanA/B* Test Result

| **BioFire BCID2 Panel Test Result** | | **Efaecium assay** | **Efaecalis assay** | **vanA/B assay** |
| --- | --- | --- | --- | --- |
| *Enterococcus faecium*  *Enterococcus faecalis*  *vanA/B* | Not Detected  Not Detected  N/A | Negative | Negative | Any result |
| *Enterococcus faecium*  *Enterococcus faecalis*  *vanA/B* | **Detected**  Not Detected  Not Detected | **Positive** | Negative | Negative |
| *Enterococcus faecium*  *Enterococcus faecalis*  *vanA/B* | Not Detected  **Detected**  Not Detected | Negative | **Positive** | Negative |
| *Enterococcus faecium*  *Enterococcus faecalis*  *vanA/B* | **Detected**  **Detected**  Not Detected | **Positive** | **Positive** | Negative |
| *Enterococcus faecium*  *Enterococcus faecalis*  *vanA/B* | **Detected**  Not Detected  **Detected**a | **Positive** | Negative | **Positive** |
| *Enterococcus faecium*  *Enterococcus faecalis*  *vanA/B* | Not Detected  **Detected**  **Detected**a | Negative | **Positive** | **Positive** |
| *Enterococcus faecium*  *Enterococcus faecalis*  *vanA/B* | **Detected**  **Detected**  **Detected**a,b | **Positive** | **Positive** | **Positive** |

a Subculturing and AST testing is required in order to assign a resistant and/or susceptible phenotype to isolates recovered from the blood culture sample.

b It is not possible to determine the species the *vanA/B* gene is associated with.

#### Table 8. Possible Assay Results and the Corresponding KPC Test Results (Example for Gram-Negative Antimicrobial Result Interpretation)

| BioFire BCID2 Panel Test Results | Acinetobacter assay | Enteric1 and/or  Enteric2 assay | Ecloacae assay | Ecoli assay | Kaerogenes assay | Koxytoca assay | Kpneumoniae assay | Proteus assay | Salmonella assay | Smarcescens assay | Paeroginosa assay | KPC Assay | Description |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Acinetobacter calcoaceticus-baumanii* complex Not Detected *Enterobacterales* Not Detected  *Enterobacter cloacae* complexNot Detected  *Escherichia coli* Not Detected  *Klebsiella aerogenes* Not Detected  *Klebsiella oxytoca* Not Detected  *Klebsiella pneumoniae* groupNot Detected  *Proteus* spp.Not Detected  *Salmonella* spp*.* Not Detected  *Serratia marcescens* Not Detected  *Pseudomonas aeruginosa* Not Detected  KPC N/A | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Any result | No applicable gram0negative bacteria detected in the sample  KPC results are not applicable (N/A) |
| More than one applicable gram-negative bacteria detected in the sample  **AND**  KPC not detected | **Pos** | **Pos** | **Pos** | **Pos** | **Pos** | **Pos** | **Pos** | **Pos** | **Pos** | **Pos** | **Pos** | Neg |  |
| ***Acinetobacter calcoaceticus-baumanii* complexDetected**  *Enterobacterales* Not Detected  *Enterobacter cloacae* complexNot Detected  *Escherichia coli* Not Detected  *Klebsiella aerogenes* Not Detected  *Klebsiella oxytoca* Not Detected  *Klebsiella pneumoniae* Not Detected  *Proteus* spp.Not Detected  *Salmonella* spp*.* Not Detected  *Serratia marcescens* Not Detected  *Pseudomonas aeruginosa* Not Detected  **KPC Detected** | **Pos** | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | **Pos** | *Acinetobacter calcoaceticus-baumanii* complex detected in the sample  **AND**  KPC detected |
| *Acinetobacter calcoaceticus-baumanii* complexNotDetected  ***Enterobacterales* Detected**  *Enterobacter cloacae* complexNot Detected  *Escherichia coli* Not Detected  *Klebsiella aerogenes* Not Detected  *Klebsiella oxytoca* Not Detected  *Klebsiella pneumoniae* Not Detected  *Proteus* spp.Not Detected  *Salmonella* spp*.* Not Detected  *Serratia marcescens* Not Detected  *Pseudomonas aeruginosa* Not Detected  **KPC Detected** | Neg | **Pos** | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | **Pos** | One or more *Enterobacterales* detected in the sample  (not *E. cloacae* complex, *E. coli*, *K. aerogenes*, *K. oxytoca*, *K. pneumoniae* group, *Proteus* spp., *Salmonella* spp., or *S. marcescens*)  **AND**  KPC detected |
| *Acinetobacter calcoaceticus-baumanii* complexNot Detected  ***Enterobacterales* Detected**  ***Enterobacter cloacae* complexDetected**  *Escherichia coli* Not Detected  *Klebsiella aerogenes* Not Detected  *Klebsiella oxytoca* Not Detected  *Klebsiella pneumoniae* Not Detected  *Proteus* spp.Not Detected  *Salmonella* spp*.* Not Detected  *Serratia marcescens* Not Detected  *Pseudomonas aeruginosa* Not Detected  **KPC Detected** | Neg | Any result | **Pos** | Neg | Neg | Neg | Neg | Neg | Neg | Neg | **Neg** | **Pos** | One or more *Enterobacterales* (including species of the *Enterobacter cloacae* complex) detected in the sample  **AND**  KPC detected |
| *Acinetobacter calcoaceticus-baumanii* complexNot Detected  ***Enterobacterales* Detected**  *Enterobacter cloacae* complexNotDetected  ***Escherichia coli* Detected**  *Klebsiella aerogenes* Not Detected  *Klebsiella oxytoca* Not Detected  *Klebsiella pneumoniae* Not Detected  *Proteus* spp.Not Detected  *Salmonella* spp*.* Not Detected  *Serratia marcescens* Not Detected  *Pseudomonas aeruginosa* Not Detected  **KPC Detected** | Neg | Any result | Neg | **Pos** | Neg | Neg | Neg | Neg | Neg | Neg | Neg | **Pos** | One or more *Enterobacterales* (including *Escherichia coli*)detected in the sample  **AND**  KPC detected |
| *Acinetobacter calcoaceticus-baumanii* complexNot Detected  ***Enterobacterales* Detected**  *Enterobacter cloacae* complexNotDetected  *Escherichia coli* NotDetected  ***Klebsiella aerogenes* Detected**  *Klebsiella oxytoca* Not Detected  *Klebsiella pneumoniae* Not Detected  *Proteus* spp.Not Detected  *Salmonella* spp*.* Not Detected  *Serratia marcescens* Not Detected  *Pseudomonas aeruginosa* Not Detected  **KPC Detected** | Neg | Any result | Neg | Neg | **Pos** | Neg | Neg | Neg | Neg | Neg | Neg | **Pos** | One or more *Enterobacterales* (including *Klebsiella aerogenes*) detected in the sample  **AND**  KPC detected |
| *Acinetobacter calcoaceticus-baumanii* complexNot Detected  ***Enterobacterales* Detected**  *Enterobacter cloacae* complexNotDetected  *Escherichia coli* NotDetected  *Klebsiella aerogenes* Not Detected  ***Klebsiella oxytoca* Detected**  *Klebsiella pneumoniae* Not Detected  *Proteus* spp.Not Detected  *Salmonella* spp*.* Not Detected  *Serratia marcescens* Not Detected  *Pseudomonas aeruginosa* Not Detected  **KPC Detected** | Neg | Any result | Neg | Neg | Neg | **Pos** | Neg | Neg | Neg | Neg | Neg | **Pos** | One or more *Enterobacterales* (including *Klebsiella oxytoca*) detected in the sample  **AND**  KPC detected |
| *Acinetobacter calcoaceticus-baumanii* complexNot Detected  ***Enterobacterales* Detected**  *Enterobacter cloacae* complexNotDetected  *Escherichia coli* NotDetected  *Klebsiella aerogenes* Not Detected  *Klebsiella oxytoca* Not Detected  ***Klebsiella pneumoniae* Detected**  *Proteus* spp.Not Detected  *Salmonella* spp*.* Not Detected  *Serratia marcescens* Not Detected  *Pseudomonas aeruginosa* Not Detected  **KPC Detected** | Neg | Any result | Neg | Neg | Neg | Neg | **Pos** | Neg | Neg | Neg | Neg | **Pos** | One or more *Enterobacterales* (including species in the *Klebsiella pneumoniae* group*)* detected in the sample  **AND**  KPC detected |
| *Acinetobacter calcoaceticus-baumanii* complexNot Detected  ***Enterobacterales* Detected**  *Enterobacter cloacae* complexNotDetected  *Escherichia coli* NotDetected  *Klebsiella aerogenes* Not Detected  *Klebsiella oxytoca* Not Detected  *Klebsiella pneumoniae* Not Detected  ***Proteus* spp.Detected**  *Salmonella* spp*.* Not Detected  *Serratia marcescens* Not Detected  *Pseudomonas aeruginosa* Not Detected  **KPC Detected** | Neg | Any result | Neg | Neg | Neg | Neg | Neg | **Pos** | Neg | Neg | Neg | **Pos** | One or more *Enterobacterales* (including *Proteus* species) detected in the sample  **AND**  KPC detected |
| *Acinetobacter calcoaceticus-baumanii* complexNot Detected  ***Enterobacterales* Detected**  *Enterobacter cloacae* complexNotDetected  *Escherichia coli* NotDetected  *Klebsiella aerogenes* Not Detected  *Klebsiella oxytoca* Not Detected  *Klebsiella pneumoniae* Not Detected  *Proteus* spp.Not Detected  ***Salmonella* spp*.* Detected**  *Serratia marcescens* Not Detected  *Pseudomonas aeruginosa* Not Detected  **KPC Detected** | Neg | Any result | Neg | Neg | Neg | Neg | Neg | Neg | **Pos** | Neg | Neg | **Pos** | One or more *Enterobacterales* (including *Salmonella* species) detected in the sample  **AND**  KPC detected |
| *Acinetobacter calcoaceticus-baumanii* complexNot Detected  ***Enterobacterales* Detected**  *Enterobacter cloacae* complexNotDetected  *Escherichia coli* NotDetected  *Klebsiella aerogenes* Not Detected  *Klebsiella oxytoca* Not Detected  *Klebsiella pneumoniae* Not Detected  *Proteus* spp.Not Detected  *Salmonella* spp*.* Not Detected  ***Serratia marcescens* Detected**  *Pseudomonas aeruginosa* Not Detected  **KPC Detected** | Neg | Any result | Neg | Neg | Neg | Neg | Neg | Neg | Neg | **Pos** | Neg | **Pos** | One or more *Enterobacterales* (including *Serratia marcescens*)detected in the sample  **AND**  KPC detected |
| *Acinetobacter calcoaceticus-baumanii* complexNot Detected  ***Enterobacterales* Detected**  *Enterobacter cloacae* complexNotDetected  *Escherichia coli* NotDetected  *Klebsiella aerogenes* Not Detected  *Klebsiella oxytoca* Not Detected  *Klebsiella pneumoniae* Not Detected  *Proteus* spp.Not Detected  *Salmonella* spp*.* Not Detected  *Serratia marcescens* Not Detected  ***Pseudomonas aeruginosa* Detected**  **KPC Detected** | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | **Pos** | **Pos** | *Pseudomonas aeruginosa* detected in the sample  **AND**  KPC detected |

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 (see Table 10). Detection of *Staphylococcus aureus* and positive *mecA/C* and MREJ results is indicative of methicillin-resistant *Staphylococcus aureus* (MRSA).

#### Table 9. Assay and Results Interpretation for the *mecA/C* Test Result

| **BioFire BCID2 Panel Test Result** | | **Sepidermidis assay** | **Slugdunensis assay** | **mecA/C**  **assay** |
| --- | --- | --- | --- | --- |
| *Staphylococcus epidermidis*  *Staphylococcus lugdunensis*  *mecA/C* | Not Detected  Not Detected  N/A | Negative | Negative | Any result |
| *Staphylococcus epidermidis*  *Staphylococcus lugdunensis*  *mecA/C* | **Detected**  Not Detected  Not Detected | **Positive** | Negative | Negative |
| *Staphylococcus epidermidis*  *Staphylococcus lugdunensis*  *mecA/C* | Not Detected  **Detected**  Not Detected | Negative | **Positive** | Negative |
| *Staphylococcus epidermidis*  *Staphylococcus lugdunensis*  *mecA/C* | **Detected**  **Detected**  Not Detected | **Positive** | **Positive** | Negative |
| *Staphylococcus epidermidis*  *Staphylococcus lugdunensis*  *mecA/C* | **Detected**  Not Detected  **Detected**a | **Positive** | Negative | **Positive** |
| *Staphylococcus epidermidis*  *Staphylococcus lugdunensis*  *mecA/C* | Not Detected  **Detected**  **Detected**a | **Negative** | **Positive** | **Positive** |
| *Staphylococcus epidermidis*  *Staphylococcus lugdunensis*  *mecA/C* | **Detected**  **Detected**  **Detected**a,b | **Positive** | **Positive** | **Positive** |

a Subculturing and AST testing is required in order to assign a resistant and/or susceptible phenotype to isolates recovered from the blood culture sample.

b It is not possible to determine the species with which the *mecA/C* gene is associated.

#### Table 10. Possible *Staphylococcus aureus* Results and *mecA/C* and MREJ Assay Combinations for the *mecA/C* and MREJ (MRSA) Result

| **BioFire BCID2 Panel Test Result** | | **Saureus**  **assay** | **mecA/C**  **assay** | **MREJ**  **assay** |
| --- | --- | --- | --- | --- |
| *Staphylococcus aureus*  *mecA/C* and MREJ (MRSA) | Not Detected  N/A | Negative | Any Result | Any Result |
| *Staphylococcus aureus*  *mecA/C* and MREJ (MRSA) | **Detected**  **Detected** a | **Positive** | **Positive** | **Positive** |
| *Staphylococcus aureus*  *mecA/C* and MREJ (MRSA) | **Detected**  Not Detected | **Positive** | Negative | Negative |
| *Staphylococcus aureus*  *mecA/C* and MREJ (MRSA) | **Detected**  Not Detected | **Positive** | **Positive** | Negative |
| *Staphylococcus aureus*  *mecA/C* and MREJ (MRSA) | **Detected**  Not Detected | **Positive** | Negative | **Positive** |

a Subculturing and AST testing is required in order to assign a resistant and/or susceptible phenotype to isolates recovered from the blood culture sample.

**NOTE: It is possible for the BioFire BCID2 Panel to report both the mecA/C result and the mecA/C and MREJ (MRSA) result if Staphylococcus epidermidis and/or Staphylococcus lugdunensis are detected in the same sample as Staphylococcus aureus.**

### Results Interpretation for Yeast

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.

Additional information about detection of specific strains, isolates, or serotypes/genotypes of yeast is provided in the Analytical Reactivity (Inclusivity) section (Table 123 – Table 129). Based on *in silico* analysis and empirical testing, each assay is specific for detection of the indicated species with the exceptions described below and in the Analytical Specificity (Cross-Reactivity and Exclusivity) section (Table 130 and Table 131).

* *Candida glabrata* is closely related to *Candida bracarensis and Candida nivarienses*, and they have been described together as the *Candida glabrata* complex. Misidentification of these species by laboratory methods does occur, and cross-reactivity between the Cglabrata assay and *C. bracarensis* or *C. nivariensis* may be observed.
* At positive blood culture levels, the Ckrusei assay may cross-react with related species of *Candida, Pichia, or Issatchenkia,* including *Candida inconspicua* and *Pichia* (*Candida*) *norvegensis*.

**NOTE: Candida krusei is also known as Issatchenkia orientalis and Pichia kudriavzevkii.**

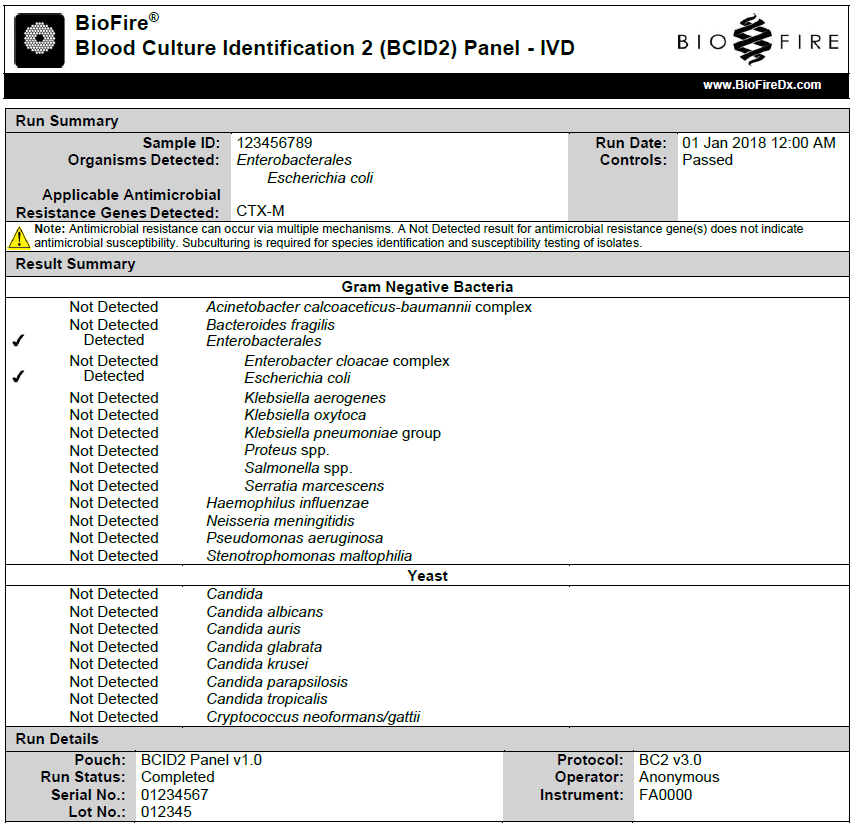
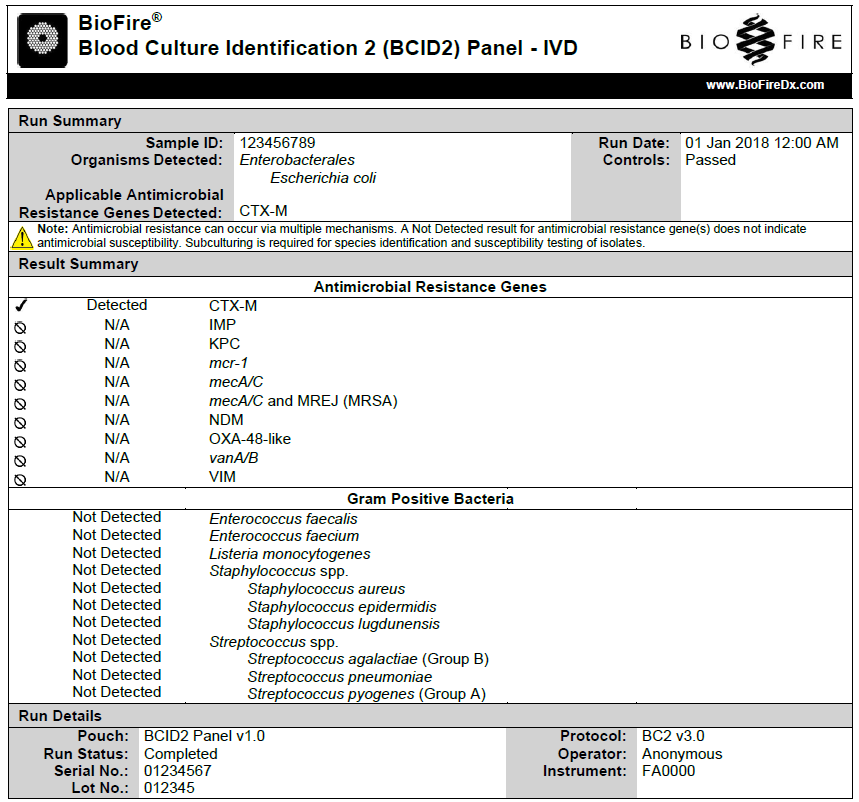
* The assay for detection of *C. parapsilosis* cross-reacts with *Candida tropicalis* at high concentration, and the assay for detection of *C. tropicalis* can cross-react with *C. parapsilosis* at high concentration. Either cross-reactive interaction would produce a dual result of *Candida parapsilosis* Detected and *Candida tropicalis* Detected.
* The Cryptococcus assay (*Cryptococcus neoformans/gattii*) can cross-react with environmental or insect-associated species of *Cryptococcus* that are not implicated in human infection.

**NOTE: Assays for the detection of Candida albicans, Candida glabrata, Candida parapsilosis, and Candida tropicalis amplify gene targets within the mitochondrial genome, and the panel will not be able to detect “petite” strains that have lost their mitochondrial DNA.**

## BioFire BCID2 Panel Test Report

The BioFire BCID2 Panel two-page test report (Figure 1) is automatically displayed upon completion of a run and can be printed or saved as a PDF file. Each report contains a Run Summary, a Result Summary, and a Run Details section.

#### Figure 1. BioFire BCID2 Panel Example Test Report (Page 1 – on left; Page 2 –on right)



### Run Summary

The Run Summary section of the test report provides the Sample ID, time and date of the run, control results and an overall summary of the test results. Any organism with a Detected result will be listed in the corresponding field of the summary. If all of the organism assays were negative then “None” will be displayed in the Organisms Detected field. If an organism was detected and an applicable antimicrobial resistance gene assay was positive, the applicable antimicrobial resistance gene will be listed as Detected in the corresponding field of the summary. If all of the applicable antimicrobial resistance gene assays were negative then “None” will be displayed in the Applicable Antimicrobial Resistance Genes Detected field. Controls are listed as Passed, Failed, or Invalid. Table 11 provides additional information for each of the possible control field results.

Table 11. Interpretation of Controls Field on the BioFire BCID2 Panel Test Report

| ControlResult | Explanation | Action |
| --- | --- | --- |
| Passed | The run was successfully completedANDBoth pouch controls were successful. | Report the results provided on the test report |
| Failed | The run was successfully completedBUTAt 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 Summarysection 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 12 provides an explanation for each interpretation and any follow-up necessary to obtain a final result.

#### Table 12. Reporting of Results and Required Actions

| Result | Explanation | Action |
| --- | --- | --- |
| Detecteda | The run was successfully completedANDThe pouch controls were successful (Passed)ANDThe assay(s) for the organism (or antimicrobial resistance gene) were POSITIVEa | Report results. |
| Not Detected | The run was successfully completedANDThe pouch controls were successful (Passed)ANDThe assay(s) for the organism (or antimicrobial resistance gene) were NEGATIVE | Report results. |
| Invalid | The pouch controls were not successful (Failed)ORThe run did not complete successfully(Run Status displayed as: Aborted, Incomplete, Instrument Error, or Software Error) | See Table 11 for instruction |
| N/A(Antimicrobial Resistance Genes only) | The run was successfully completedANDThe pouch controls were successful (Passed)ANDThe 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. |

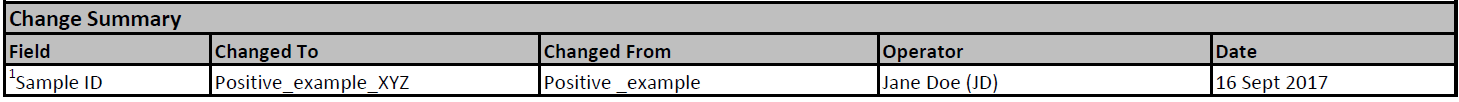
a If four or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.

### Run Details

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, 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.

### Change Summary

It is possible to edit the Sample ID once a run has completed. If this information has been changed, an additional section called **Change Summary** will be added to the test report. This Change Summary 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.



### References/Related Documents

BioFire® Blood Culture Identification 2 (BCID2) Panel CE-IVD Instructions for Use (RFIT-PRT-0841-02) BioFire Diagnostics, LLC.