PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

FilmArray® Blood Culture Identification Panel Procedure

Department of Microbiology

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1.0 Purpose and Test Principle

The FilmArray Blood Culture Identification (BCID) Panel is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with the FilmArray Instrument. The BCID Panel is capable of simultaneous detection and identification of multiple bacterial and yeast nucleic acids and select genetic determinants of antimicrobial resistance. The BCID assay is performed directly on positive blood culture samples that demonstrate the presence of organisms as determined by Gram stain. The following gram-positive bacteria, gram-negative bacteria, and yeast are identified using the BCID Panel: *Enterococcus*, *Listeria monocytogenes*, *Staphylococci* (including specific differentiation of *Staphylococcus aureus)*, *Streptococci* (with specific differentiation of *S. agalactiae*, *S. pneumoniae*, and *S. pyogenes*), *Acinetobacter baumannii*, *Enterobacteriaceae* (including specific differentiation of the *Enterobacter cloacae* complex, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Proteus,* and *Serratia marcescens*), *Haemophilus influenzae*, *Neisseria meningitidis*, *Pseudomonas aeruginosa*, *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis*. The BCID Panel also contains assays for the detection of genetic determinants of resistance to methicillin (*mecA*), vancomycin (*vanA* and *vanB*), and carbapenems (bla_{KPC}) to aid in the identification of potentially antimicrobial resistant organisms. The antimicrobial resistance gene detected may or may not be associated with the agent responsible for disease. Negative results for these select antimicrobial resistance gene assays do not indicate susceptibility, as multiple mechanisms of resistance to methicillin, vancomycin, and carbapenems exist.

The BCID panel is a closed, disposable device that houses all the chemistry required to isolate, amplify and detect nucleic acid from multiple bloodstream pathogens within a single blood culture sample. The rigid plastic component (fitment) of the BCID pouch contains reagents in freeze-dried form. The flexible plastic portion of the pouch is divided into discrete segments (blisters) where the required chemical processes are carried out. The user loads the sample into the BCID pouch, places the pouch into the FilmArray Instrument, and starts the run. All other operations are automated.

The following is an overview of the testing procedure:

1. Remove the pouch from its vacuum-sealed package. Since solutions are drawn into the pouch by vacuum, it is important to keep pouches in their protective packaging until the time of use.

2. Place the pouch into the Loading Station. The Loading Station has been designed to prevent error by providing instructions and visual cues in the form of color-coded arrows to ensure that the pouch is properly loaded.
3. Load Hydration Solution into the pouch using the Pouch Hydration Syringe. The syringe is fitted with a blunt stainless steel cannula, which is used to deliver the solution into the pouch. Loading the pouch with Hydration Solution rehydrates the freeze-dried reagents contained in the pouch fitment.
4. Remove blood culture media from the bottle using a syringe with a blunt needle and add it to the Sample Buffer vial. Mix with Transfer Pipette. The Sample Buffer contains reagents that promote binding of nucleic acids to magnetic beads for isolation.
5. Load the sample/buffer mixture into the pouch using the Sample Loading Syringe. When the sample mixture is loaded, a process control contained in the fitment of the pouch is introduced into the sample. The process control monitors all of the critical processes that occur in the pouch.
6. Transfer the pouch to the instrument and initiate a run. The FilmArray Instrument Control application provides onscreen animations illustrating the steps needed to start the run.

The following is an overview of the operations and processes that occur during a FilmArray run:

1. **Nucleic Acid Purification** - Nucleic acid purification occurs in the first three blisters of the pouch. The sample is lysed by agitation (bead beating) and the liberated nucleic acid is captured, washed and eluted using magnetic bead technology. These steps require about ten minutes and the bead-beater apparatus can be heard as a high-pitched whine during the first minute of operation.
2. **1st Stage Multiplex PCR** - The purified nucleic acid solution is combined with a preheated master mix to initiate thermocycling for multiplex PCR. The effect of 1st stage PCR is to enrich for the target nucleic acids present in the sample.
3. **2nd Stage PCR** - The products of first stage PCR are diluted and mixed with fresh PCR reagents containing a double stranded DNA binding dye (LCGreen® Plus, BioFire Diagnostics, Inc.). This solution is distributed over the 2nd stage PCR array. The individual wells of the array contain primers for different assays (each present in triplicate) that target specific nucleic acid sequences from each of the pathogens detected, as well as control template material. These primers are 'nested' or internal to the specific products of the 1st stage multiplex reaction, which enhances both the sensitivity and specificity of the reactions.
4. **DNA Melting Analysis** – After 2nd stage PCR, the temperature is slowly increased and fluorescence in each well of the array is monitored and analyzed to generate a melt curve. The temperature at which a specific PCR product melts (melting temperature or Tm) is consistent and predictable and the FilmArray Software automatically evaluates the data from replicate wells for each assay to report results. For a description of data interpretation and reporting see the Interpretation of Results section.

The FilmArray Software controls the operation of the instrument, collects and analyzes data, and automatically generates a test report at the end of the run. The entire process takes about an hour. Additional details can be found in the FilmArray Operator's Manual.

2.0 Clinical Significance

Sepsis is defined as a systemic inflammatory response syndrome in response to infection. Sepsis is the 11th leading cause of death in the United States. Life-threatening bacterial and fungal sepsis currently strikes approximately 240 out of 100,000 people per year in the U.S., with severe sepsis (associated with acute organ dysfunction) in 95 out of 100,000 people. Timely diagnosis and administration of effective treatment can significantly reduce mortality, duration of hospital stays, and costs due to sepsis. The BCID Panel simultaneously tests a single positive blood culture sample to provide results for 24 different organisms that cause bloodstream infections and three genetic markers that are known to confer antimicrobial resistance. The test can be performed on blood culture bottles that are flagged as positive by a continuously monitoring blood culture instrument and positive by Gram stain examination. BCID Panel results are available within about one hour. Rapid identification of the organism(s) in the blood culture,

along with information about antimicrobial resistance gene status for select microorganisms, can aid physicians in making appropriate treatment decisions.

2.1 Gram-Positive Bacteria

***Enterococcus* spp.** normally inhabit the alimentary tract of humans. Infections include urinary tract infections, hepatobiliary sepsis, endocarditis, surgical wound infection, bacteremia, and neonatal sepsis. There are 28 species of *Enterococcus*. While at least 12 species have been shown to cause human disease, *E. faecalis* (80-90%) and *E. faecium* (5-15%) cause the majority of clinical infections. Enterococci can carry vancomycin resistance genes such as *vanA/B*.

Listeria monocytogenes is ubiquitous in soil and water and can be found in the gastrointestinal tract of up to 5% of healthy human adults. Listeriosis is considered the most severe bacterial foodborne infection due to its high mortality rate despite early antibiotic treatment (11-60%). Populations at risk for developing invasive listeriosis include the immunosuppressed, pregnant women, neonates, fetuses and the elderly. Invasive listeriosis can result in abortion, sepsis, and meningoencephalitis. Septicemia can account for greater than 50% of cases and can have a mortality rate up to 70% when associated with severe underlying conditions.

***Staphylococcus* species** colonize the skin and mucous membranes. They are opportunistic pathogens that can cause infection following breaks in the cutaneous epithelial barrier through trauma or medical interventions. Diagnostically, the genus is divided between coagulase-positive staphylococci and coagulase-negative staphylococci (CoNS). The most clinically-relevant *Staphylococcus* is the coagulase-positive *S. aureus*. Other coagulase-positive species, such as *S. intermedius*, are isolated much less frequently from clinical specimens. *S. aureus* is the most common cause of nosocomial skin and soft tissue infections, and is second only to CoNS as a cause of primary bacteremia in hospitals. CoNS species are isolated very regularly from clinical specimens and care must be taken to assess clinical significance to differentiate between contamination, colonization, and true infection. The primary mediator of methicillin resistance in staphylococci is acquisition of the *mecA* gene.

***Streptococcus* species** are frequently found as commensal bacteria on mucous membranes, and are occasionally present as transient skin microbiota. Streptococci have historically been grouped as beta-hemolytic or non-beta-hemolytic, and also divided according to presence of specific surface antigens (i.e., Lancefield grouping). Lancefield groups A, B, C, and G are pyogenic and most are also beta-hemolytic. Of these, the Group A streptococci (represented primarily by *S. pyogenes*) and the Group B streptococci (*S. agalactiae*) are the most common in cases of septicemia. The non-pyogenic streptococci are subdivided into five groups (Mitis, Anginosus, Salivarius, Mutans, and Bovis groups). The Mitis, Anginosus, and Salivarius groups are also referred to as viridians streptococci; these bacteria produce no Lancefield antigens and are alpha-hemolytic or nonhemolytic. Viridans streptococci are also fairly common agents of septicemia causing 0.5% of sepsis cases in nonneutropenic patients and up to 2% in neutropenic patients. *S. pneumoniae* has been classified into the Mitis group but is often considered as its own separate group.

Streptococcus pyogenes (Group A *Streptococcus*) colonizes the human skin and upper respiratory tract, with these sites serving as primary focal sites of infections and principal reservoirs of transmission. *S. pyogenes* possesses complex virulence mechanisms to avoid host defenses and is responsible for deep or invasive infections, especially bacteremia, sepsis, and deep soft tissue infections.

Streptococcus agalacticae (Group B *Streptococcus* or GBS) can cause both early onset neonatal disease, characterized by sepsis and pneumonia within the first seven days of life; and late onset disease with meningitis and sepsis between day seven and three months of age. In adult patients, the spectrum of *S. agalacticae* infections includes bacteremia, pneumonia, meningitis, and endocarditis.

Streptococcus pneumoniae colonizes the upper respiratory tract, and is the most frequently isolated respiratory pathogen in community-acquired pneumonia. It is also a major cause of meningitis in pediatric and adult patients.

2.2 Gram-Negative Bacteria

Acinetobacter baumannii is a ubiquitous, non-fermentative gram-negative coccobacillus that primarily acts as an opportunistic pathogen infecting critically-ill patients. While hospital-acquired pneumonia is the most common infection caused by *A. baumannii*, other nosocomial infections are increasing in frequency, including bacteremia, skin and soft tissue infections, urinary tract infections, and infections involving the central nervous system. The bacteria are capable of persisting for long periods on environmental surfaces, aiding the spread of infections in hospital settings. Additionally, the rapid emergence of multi-drug resistant strains creates difficulties for treatment and infection control. MDR strains demonstrate resistance to most antibiotic classes, including carbapenems. Various carbapenem-hydrolyzing metallo-β-lactamases may be carried by the bacteria. The specific carbapenemase, KPC, has also been infrequently identified in *A. baumannii.* Several related *Acinetobacter* species cannot be reliably differentiated from *A. baumannii* by some manual or automated phenotypic microbial identification systems. These species include *A. calcoaceticus, A. pittii*, and *A. nosocomialis*. Together with *A. baumannii*, these species have been grouped into the *Acinetobacter calcoaceticus-baumannii* complex.

Enterobacteriaceae is composed of many genera and species of bacteria that share common features. They are widely distributed in the environment and are found on plants, in soil and water, and within the gastrointestinal tract of many animals and humans. While many organisms in this family are harmless, several are medically important and are associated with bacteremia and other illnesses, particularly gastroenteritis. Together, members of the *Enterobacteriaceae* family are among the most commonly recognized organisms seen in healthcare-associated infections. The BCID Panel contains specific assays for the *Enterobacteriaceae* members that are most frequently associated with bacteremia and/or are associated with a higher frequency of antimicrobial resistance: *Enterobacter cloacae* complex*, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus,* and *Serratia marcescens*. However, many other *Enterobacteriaceae* can also cause bacteremia, though less frequently. These include *Cedecea, Citrobacter, Pantoea, Salmonella,* and *Enterobacter aerogenes*, among others. The emergence and spread of antimicrobial resistance in *Enterobacteriaceae* has increased the complexity of treating sepsis due to these organisms. Resistance to third and fourth-generation cephalosporins is mediated primarily by production of extended-spectrum β-lactamases and overproduction of AmpC β-lactamases. While the majority of *Enterobacteriaceae* remain susceptible to carbapenems, KPC-type carbapenemases are emerging and spreading in *Enterobacteriaceae* certain locations within the United States and worldwide.

***Enterobacter cloacae* complex** are members within the *Enterobacteriaceae* family. These bacteria are ubiquitous in the environment. Clinical significance of *Enterobacter* is primarily limited to *E. cloacae* and *E. aerogenes*, which are common opportunistic nosocomial pathogens. The range of infections caused by *Enterobacter* spp. includes respiratory tract infections, urinary tract infections, soft tissue infections, septic arthritis, osteomyelitis, and bacteremia among others. *Enterobacter* spp. are estimated to be the fourth most common etiological agents in gram-negative bloodstream infections. They often have the ability to overproduce AmpC β-lactamases and are increasingly found to carry extended spectrum β-lactamases. Several species that are closely related to *E. cloacae* are grouped together with it in the *E. cloacae* complex; these include *E. asburiae, E. hormaechei*, *E. kobei*, *E. ludwigii*, and *E. nimipressuralis*.

Escherichia coli is an enteric organism most frequently isolated from the intestines of humans and animals. While most pathogenic *E. coli* infections are associated with gastrointestinal illness, certain strains may cause extraintestinal infections in healthy as well as immunocompromised individuals. These include urinary tract infections, bacteremia, and meningitis. As with other *Enterobacteriaceae*, extended spectrum β-lactamases pose a significant antibiotic resistance problem.

Klebsiella are ubiquitous in the environment, particularly in agricultural settings, and may colonize the skin, respiratory, and gastrointestinal tracts of humans. They are opportunistic pathogens and while colonization does not always result in illness, infection rates in carriers are four times higher than non-carriers. *K. pneumoniae* and *K. oxytoca* are the species most

frequently isolated from hospitalized patients. Infections due to these bacteria include soft tissue infections, urinary tract infections, pneumonia, and septicemia. Both *K. pneumoniae* and *K. oxytoca* can carry the carbapenemase gene, *bla*$_{KPC}$, which makes them resistant to carbapenem antibiotics. Biochemical discrimination between species of *Klebsiella* is difficult: *K. oxytoca* isolates may be erroneously identified as *K. pneumoniae* by manual or automated biochemical detection algorithms. *Raoultella ornithinolytica*, which was recently separated from the genus *Klebsiella*, can also be misidentified as *K. oxytoca* by phenotypic identification systems.

Proteus species are commonly isolated in the clinical laboratory, with *Proteus mirabilis* being the most frequently seen species. Most infections (approximately 85%) are thought to be community acquired; however, nosocomial outbreaks have also occurred. The majority of *P. mirabilis* isolates are isolated from complicated urinary tract infections involving abnormalities or indwelling catheters, while other species (e.g., *P. vulgaris*) are more commonly found in soft tissue infections. Progression of these infections to septicemia is relatively uncommon.

Serratia marcescens is seldom a cause of primary infections, but is a notorious nosocomial pathogen and colonizer. It is of particular concern due to its emerging antibiotic resistance to commonly used agents like β-lactams, aminoglycosides, carbapenems, and fluoroquinolones. Transmission may occur from person to person contact, via medical apparatus, intravenous fluids, or other solutions. Patients with indwelling catheters, particularly those for urinary tract infections, serve as a primary reservoir for transmission via hospital personnel.

Haemophilus influenzae is isolated exclusively from humans. Strains of *H. influenzae* are divided into two groups based on the presence or absence of a capsular polysaccharide. Encapsulated strains are further divided into six serotypes (a through f). Prior to widespread use of the *H. influenzae* type b (Hib) conjugate vaccines, Hib caused >80% of invasive *H. influenzae* infections, predominantly in children under the age of five, with a mortality rate of 3 to 6% and a further 20 to 30% developing permanent sequelae ranging from mild hearing loss to mental retardation. In areas of routine vaccination, the majority of invasive *H. influenzae* infection is caused by nontypeable strains and predominantly affects children under the age of one and the elderly, with a mortality rate of 13 to 20%.

Neisseria meningitidis is spread by mucus or respiratory droplets often from asymptomatic carriers. Thirteen different serogroups of *N. meningitidis* (A, B, C, D, H, I, K, L, X, Y, Z, W135, and 29E) can be distinguished. Serogroups B, C, and Y are currently the most prevalent in developed countries and serogroup A is predominant in the rest of the world. Serogroups W135 and X also cause epidemics in developing regions of the world. The serogroups are determined by a polysaccharide capsule that aids bacterial survival inside the human host. Meningococcal disease (spinal meningitis and/or meningococcemia) is rare in developed countries, but can occur in outbreaks. It is most common in infants, children, and young adults, and appears in places with crowded living conditions (e.g., college dormitories and military barracks). Seasonal incidence peaks in late winter and early spring. Septicemia with *N. meningitidis* is associated with fever and a characteristic hemorrhagic rash that may be transient. The disease can progress extremely quickly (< 24 h) with hypotension, multiorgan dysfunction, shock, peripheral ischemia, and limb loss and has a mortality rate of approximately 5-10%.

Pseudomonas aeruginosa is an opportunistic pathogen. It rarely causes disease in healthy individuals but can cause sepsis in patients with burn wounds, malignancies or immunodeficiency, or in preterm infants. *P. aeruginosa* is a leading cause of nosocomial infections and is responsible for 10% of all hospital acquired infections. Mortality rates due to *P. aeruginosa* bacteremia are greater than 20%, and may be as high as 50% for intensive care unit patients and burn victims. *P. aeruginosa* is susceptible to a limited number of antibiotics (antipseudomonal penicillins and cephalosporins, carbapenems, fluorquinolones and ciprofloxacin), and multi-drug resistant *P. aeruginosa* infection is becoming an increasing problem in hospitals. The carbapenemase, KPC, has recently been identified in isolates of *P. aeruginosa*.

2.3 Yeast

***Candida* species** are yeasts that are ubiquitous in the environment and as members of the normal human microbiota, especially in the digestive tract and on mucous membranes. These fungi are important causative agents of opportunistic nosocomial infections ranging from superficial to systemic. *Candida* spp. are the 4th most common cause of nosocomial bloodstream infections. The mortality rate for *Candida* bloodstream infection is approximately 40% and they often occur in combination with bacteria or a second *Candida* species. The five most common species causing bloodstream infections are *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*. Species distribution has changed in the past three decades so that non-*C. albicans* have become more frequent than *C. albicans*. This shift is significant due to non-*C. albicans* species, especially *C. glabrata* and *C. krusei*, having increased rates of resistance to fluconazole, the drug most often used to treat *Candida* bloodstream infections. Less common *Candida* species may be misidentified as one of the five common species using phenotypic laboratory testing. In particular, *C. dubliniensis* can be misidentified as *C. albicans;* and *C. orthopsilosis* and *C. metapsilosis* can be misidentified as *C. parapsilosis*.

2.4 Antimicrobial Resistance Genes

mecA – Methicillin resistant Staphylococcus are a concern in both hospital-acquired and community-acquired infections. Few options exist for treatment of these infections, as the bacteria are resistant to both natural and semi-synthetic β-lactam antibiotics. The primary mechanism of methicillin resistance in staphylococci is through acquisition of the *mecA* gene that encodes a penicillin binding protein (PBP2a) that has low affinity for β-lactams. *mecA* is part of a gene complex carried on a chromosomally-integrated mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*). Ten major SCC*mec* types (I - X) have been characterized. In 2011, a *SCCmec* type XI cassette carrying a divergent *mecA* homologue (*mecA*$_{LGA251}$/*mecC*), which also confers methicillin resistance, was identified. Although coagulase-negative staphylococci (CoNS) have higher rates of methicillin resistance (MR-CoNS) than *S. aureus* (MRSA), CoNS is less virulent than *S. aureus* and is primarily limited to infections in the immunocompromised or individuals with indwelling devices. Other mechanisms, such as penicillin binding protein mutations and hyperproduction of staphylococcal β-lactamase, can facilitate reduced methicillin susceptibility in *S. aureus* in the absence of *mecA*.

vanA/B – Vancomycin resistant Enterococcus has increased rapidly, accounting for 60% of *E. faecium* and 2% of *E. faecalis* isolated from the bloodstream. Infection with a VRE increases the risk of death to 75%, compared with 45% for infection with a susceptible strain. Eight gene clusters associated with vancomycin resistance have been identified to date (*vanA*, *vanB*, *vanC*, *vanD*, *vanE*, *vanG*, *vanL*, and *vanM*), with *vanA* and *vanB* being the most common in clinical isolates. Both the *vanA* and *vanB* gene clusters are borne on mobile genetic elements (transposons) and can be located either on the chromosome or carried on a plasmid. Enterococci carrying *vanA* or *vanB* are resistant to high levels of vancomycin. The next most commonly detected Enterococci, *E. gallinarium* and *E. casseliflavus*, display intrinsic low-level resistance (intermediate resistance, *vanC* phenotype) to vancomycin in the absence of *vanA/B*.

KPC – *Carbapenem resistant Enterobacteriaceae* (CRE) are increasingly important pathogens in the hospital setting. Limited treatment options exist for CRE and they are associated with high mortality rates. Those most at risk include patients receiving long courses of antibiotics and those with indwelling devices (e.g. ventilators, urinary catheters, or intravenous catheters). The most common mechanism of carbapenemase-resistance in CRE in the United States is that conferred by *Klebsiella pneumoniae* carbapenemases (KPCs) genes (*bla*$_{KPC}$), a family of carbapenemase enzymes first described in 2001. Since their emergence, KPCs have become endemic in several countries. Twelve variants have been identified to date, KPC-2 through KPC-13, which differ by one to three amino acids. KPCs are frequently carried on mobile genetic elements with the potential to spread between organisms. KPCs have been identified in many *Enterobacteriaceae*, the most common being *K. pneumoniae.* In addition to CRE, non-*Enterobacteriaceae* such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* may also harbor KPCs. However, KPCs are not the most common mechanisms of carbapenem resistance

in these two organisms. *P. aeruginosa* and *A. baumannii* isolates frequently carry other β-lactamases that confer carbapenem resistance (e.g. VIM, IMP, SIM, OXAs) or have porin downregulation leading to carbapenem resistance. Detection of KPCs using phenotypic susceptibility testing (e.g., MIC breakpoints or Modified Hodge Test) is very difficult, not only because other mechanisms of carbapenem-resistance exist, but also because KPC activity is regulated by multiple mechanisms that may not be accurately assessed *in vitro* resulting in incorrect susceptibility reporting. Alternatively, molecular methods (e.g., PCR) are increasingly being used to specifically identify KPC genes in clinical isolates.

3.0 Scope

This procedure is classified under CLIA as Moderately Complex. It should be carried out by technical personnel familiarized and trained on the operation of the FilmArray. Testing includes but is not limited to: instrument start up, shutdown, routine maintenance, basic troubleshooting, QC checks, technical proficiency. Records of employee proficiency testing are kept in the department. Performance reviews of technical personnel are to be carried out annually.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

The reagents and/or chemicals that are used in this procedure may be hazardous to your health if handled incorrectly. A brief listing of precautions for each chemical hazard is included in the reagent section of this procedure.

More extensive information concerning the safe handling of the reagents and/or chemicals used in this procedure, as well as other important safety information may be obtained by consulting the Material Safety Data Sheet (SDS/MSDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Blood borne pathogens
- Hazardous reagents

To perform this procedure, you must use:

- Gloves – must be worn when handling specimens and reagents.
- Laboratory Coat – must be worn when handling specimens and reagents.
- Biological Safety Cabinet – must be used when processing specimens.

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

Reference for spill/decontamination:

- SDS/MSDS
- Chemical hygiene plan

5.0 Equipment, Materials, and Reagents

5.1 Equipment

- Microscope with objectives for x40 and x1000 (oil immersion) magnification
- FilmArray Instrument & Pouch Loading Station

5.2 Reagents and Materials for Microscopic Examination & Prescreening

- 20-gauge transfer needles
- Gram stain reagents
- Glass slides

- 22 x 22 mm coverslips
- Coagulase plasma in 0.5 mL aliquots – store at ≤ 2°C until needed for use

5.3 FilmArray Blood Culture Identification Panel Kit

Each FilmArray BCID Panel Kit contains sufficient reagents to test 30 samples. Store the test kit, including reagent pouches and buffers, at room temperature (18–30°C). **DO NOT REFRIGERATE.** Always check the expiration date, and do not use reagents beyond the expiration date printed on the pouch or kit. Do not use pouches if the outer packaging has been damaged or if the vacuum is not intact. Do not remove pouches from their packaging until a sample is ready to be tested. Once the pouch packaging has been opened, the pouch should be loaded as soon as possible (within approximately 30 min). Once a pouch has been loaded, the test run should be started as soon as possible (within 60 min).

Materials Required for Sampling Positive Blood Cultures:

- 1 cc insulin syringes capable of measuring 0.1 mL (100 µL) sample volume
- 18-gauge blunt fill needles

FilmArray Kit Components:

- Individually packaged FilmArray BCID pouches
- Single-use (0.5 mL) Sample Buffer vials (red lid)
  Sample Buffer is assigned the following classifications: Acute toxicity (Category 4), Serious Eye damage (Category 1), and Skin irritation (Category 2). Accordingly, the Sample Buffer is harmful if swallowed, causes serious eye damage, and causes skin irritation. Sample Buffer will form hazardous compounds and fumes when mixed with bleach or other disinfectants. Bleach should never be added to Sample Buffer or sample waste.
- Single-use (1.5 mL) Hydration Solution Vials (blue lid)
- Individually packaged Transfer Pipettes
- Individually packaged Sample Loading Syringes with attached cannula (red cap)
- Individually packaged Pouch Hydration Syringes with attached cannula (blue cap)

5.4 Control Materials and Usage

- Pooled suspensions of control strains representing each target except *Neisseria meningitidis.* Control material should be frozen in aliquots and stored at -70°C.

6.0 Interfering Substances

The manufacturer performed studies with the FilmArray BCID Assay in the presence of a variety of potential biological and chemical substances in order to characterize the ability of the assay to detect target DNA under these conditions. A complete description of the studies can be found in the manufacture's package insert. Results demonstrated no reportable interference or inhibition with any substances tested.

7.0 Warnings and Precautions

7.1 Preventing Organism Contamination

Due to the sensitive nature of the FilmArray BCID Panel, it is important to guard against contamination of the work area by following these guidelines:

- Positive blood culture samples contain high concentrations of organisms and require careful adherence to the sample processing steps described in this procedure. To avoid possible contamination, samples should be processed in a biosafety cabinet.
- Prior to processing samples, thoroughly clean both the work area and the FilmArray Pouch Loading Station using a suitable cleaner such as freshly prepared 10% bleach or a similar disinfectant. To avoid residue buildup and potential PCR inhibition, wipe disinfected surfaces with water.
- Samples and pouches should be handled one-at-a-time.
- Change gloves and clean the work area between each sample.

7.2 Preventing Amplicon Contamination

A common concern with PCR-based assays is false positive results caused by contamination of the work area with PCR amplicon. Because the FilmArray BCID Panel pouch is a closed system, the risk of amplicon contamination is low provided that pouches remain intact after the test is completed. Adhere to the following guidelines to prevent amplicon contamination:

- Discard used pouches in an appropriate biohazard container immediately after the run has completed.
- Avoid excessive handling of pouches after test runs.
- Avoid exposing pouches to sharp edges or anything that might cause a puncture.
- Never wear gloves or lab coats worn in amplification/detection area, where the instrument is located, out into the main lab.
- When unloading completed test pouches, examine the exterior of the film for drips or liquid leaking.

WARNING: If liquid is observed on the exterior of a pouch, the liquid and pouch should be immediately contained and discarded in a biohazard container. The instrument and work space must be decontaminated as described in the FilmArray Operator's Manual. DO NOT PERFORM ADDITIONAL TESTING UNTIL THE AREA HAS BEEN DECONTAMINATED.

8.0 Sample Requirements

Sample Volume – 0.1 mL

Sample Age – Blood culture samples should be processed and tested as soon as possible after being flagged as positive by the culture instrument. However, samples may be stored for up to 8 hours at room temperature or in the culture instrument prior to testing.

9.0 Procedure

9.1 Gram Stain & Prescreening

A 20-gauge transfer needle should be used to access the contents of the positive blood culture bottle. Prepare smears for Gram stain as described in the Blood Culture Procedure. The FilmArray BCID Panel is performed directly on positive blood culture samples that demonstrate the presence of organisms as determined by Gram stain. Unless subsequent positive bottles produce different organism morphologies, only the first positive blood culture bottle should be tested with the FilmArray assay.

Due to the significant cost associated with the performance of the FilmArray assay, blood cultures which demonstrate Gram-positive cocci resembling staph and Gram-positive rods should be screened prior to testing to reduce wasted resources associated with the work-up of contaminating bacteria. Blood cultures demonstrating any other organism morphology can be tested automatically.

9.1.1 Gram-Positive Cocci Resembling Staph

All bottles that demonstrate Gram-positive cocci resembling staph should be screened with a tube-coagulase prior to testing with the FilmArray.

Tube Coagulase Procedure

1. Remove a 0.5 mL aliquot of plasma from the -20°C freezer and bring to room temperature.
2. Place an accession label corresponding to the blood culture bottle on the vial without obstructing the view of the plasma.
3. Inoculate the plasma with 2 drops of the positive blood culture. Do not over inoculate.
4. Incubate the inoculated tube of plasma at 35°C without CO_2 for up to 4 h and observe for clot formation hourly. Gently tilt (do not agitate) the tube during observations.

5. If any degree of clot formation is observed within 4 h, proceed with FilmArray testing. If no clot formation is observed, do not perform FilmArray testing. Proceed with subculture and identification as indicated in the Blood Culture Procedure.

9.1.2 Gram-Positive Rods

All bottles that demonstrate regular, small Gram-positive rods should be screened with a motility test prior to testing with the FilmArray. Small Gram-positive rods that are motile may be *Listeria monocytogenes* and warrant FilmArray testing. However, non-motile rods are most likely *Corynebacterium* spp., which are often skin contaminants. Bottles that demonstrate large Gram-positive rods resembling *Bacillus* spp. should not be tested with the FilmArray.

Motility Test Procedure

1. Place 1 drop of the positive blood culture onto a glass slide.
2. Apply a 22 x 22 mm coverslip to the sample and examine microscopically using the high dry objective (for a magnification of x 400).
3. If organisms appear motile, proceed with FilmArray testing. *Listeria monocytogenes* cells typically move end over end and are described as having a "tumbling motility." If no motility is observed, do not perform FilmArray testing. Proceed with subculture and identification as indicated in the Blood Culture Procedure.

9.2 Pouch Preparation

1. Thoroughly clean the work area and the FilmArray Pouch Loading Station with freshly prepared 10% bleach (or suitable disinfectant) followed by a water rinse.
2. Remove the pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective aluminum canister.
   NOTE: If the vacuum seal of the pouch is not intact, the pouch may still be used. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.
3. Place an accession label near the barcode label on the pouch. Do not cover the barcode on the pouch.
4. Slide the pouch into the Pouch Loading Station so that the red and blue labels on the pouch align with the red and blue arrows on the base of the FilmArray Pouch Loading Station.
5. Place a blue-capped Hydration Solution vial in the blue well of the Pouch Loading Station.
6. Place a red-capped Sample Buffer vial in the red well of the Pouch Loading Station.

9.3 Pouch Hydration

1. Remove the blue-labeled Pouch Hydration Syringe from the packaging. If the cannula/tip is not firmly attached to the syringe, hold the capped tip and rotate the syringe to tighten.
2. Using the Pouch Hydration Syringe, draw Hydration Solution to at least the 1 mL mark on the syringe, taking care to avoid the formation of bubbles. If you notice bubbles at the base of the syringe, leave the tip of the cannula in the Hydration Solution vial and dislodge the bubbles by gently tapping the side of the syringe with your finger. The bubbles will float up to the plunger. **DO NOT remove air bubbles by inverting the syringe and expelling liquid.**
3. Insert the cannula tip into the port in the pouch fitment located directly below the blue arrow of the FilmArray Pouch Loading Station. While holding the body of the syringe, push down forcefully in a firm and quick motion until you hear a faint "pop" and feel an ease in resistance. The correct volume of liquid will be pulled into the pouch by vacuum; there is no need to use the plunger. **DO NOT push the syringe plunger. Injecting liquid will cause the pouch to overfill.**
4. Verify that the pouch has been hydrated. **If the Hydration Solution is not automatically drawn into the pouch, discard the current pouch and start from the beginning with a new pouch.** Most of the liquid will have been drawn out of the syringe. Also, check to see that fluid has entered and hydrated reagents in the reagent wells (eleven wells located at the base of the rigid plastic part of the pouch). Flip the barcode label down to see the reagent

wells. Small air bubbles may be seen. If the pouch fails to hydrate (dry reagents appear as white pellets), repeat Step 4 to verify that the seal of the port was broken or retrieve a new pouch and repeat from Step 2 of the Pouch Preparation section.

9.4 Prepare Sample Mix

1. Invert the positive blood culture bottle several times to mix.
2. Wipe the bottle septum with alcohol and air dry.
3. Tilt the bottle and use a syringe with a blunt 18-gauge needle to withdraw 0.1 mL of blood culture sample through the bottle septum, taking care to avoid the formation of bubbles.
4. Transfer sample directly to the Sample Buffer vial. Discard syringe in an appropriate biohazard sharps container. Alternately: Draw the desired amount of blood culture sample (> 0.1 mL) from the bottle into the syringe and transfer to a sterile secondary container. Draw the blood culture sample from the secondary container to the first line of the Transfer Pipette (0.1 mL) and add the sample to the Sample Buffer vial.
5. Use the pipette to mix the sample with the Sample Buffer by gently pipetting up and down.

9.5 Load Sample Mix

1. Remove the red-labeled Sample Loading Syringe from the packaging. If the cannula/tip is not firmly attached to the syringe, hold the capped tip and rotate the syringe to tighten.
2. Using the Sample Loading Syringe, draw approximately 0.3 mL of sample/buffer mix (to the 0.3 mL/cc mark on the syringe), taking care to avoid the formation of bubbles. If you notice bubbles at the base of the syringe, leave the tip of the cannula in the Sample Buffer vial and dislodge the bubbles by gently tapping the side of the syringe with your finger. The bubbles will float up to the plunger. **To avoid contaminating the work area, do not remove air bubbles by inverting the syringe and expelling liquid.**
3. Insert the cannula tip into the port in the pouch fitment located directly below the red arrow of the FilmArray Pouch Loading Station. While holding the body of the syringe, push down forcefully in a firm and quick motion until you hear a faint “pop” and feel an ease in resistance. The correct volume of liquid will be pulled into the pouch by vacuum; **DO NOT push the syringe plunger**.
4. Verify that the sample has been loaded. Most of the liquid will have been drawn out of the syringe. 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 Loading Syringe, the pouch should be discarded. Retrieve a new pouch and repeat from Step 2 of the Pouch Preparation section.
5. Dispose of syringes in an appropriate biohazard sharps container. **To reduce risk of exposure to hazardous or potentially infectious material, do not re-cap the syringes.**
6. Remove the pouch from the loading station and carry it to the FilmArray instrument. Remove lab coat and gloves before entering the room with the FilmArray instruments. A new pair of gloves should be donned once inside the testing area. It is not necessary to wear a lab coat while in the FilmArray testing area.

9.6 Run Pouch

The FilmArray Instrument Control Software includes a step-by-step on-screen tutor that shows each step of the test.

1. Ensure that the laptop and FilmArray Instrument have been turned on. Launch the FilmArray Instrument Control Software by double clicking on the desktop icon.
2. Open the instrument lid.
3. Insert the loaded FilmArray pouch into the instrument.
   Position the pouch so that the array is on the right and the film is inserted first. The red and blue labels on the FilmArray pouch should align with the red and blue arrows on the FilmArray Instrument. There is a ‘click’ when the FilmArray pouch has been placed securely in the instrument. If inserted correctly, the pouch barcode is visible. If the FilmArray pouch is not completely in place, the instrument will not continue to the next step. **If the pouch does**

not slide into the instrument easily, gently push the lid of the instrument back to be sure that it is completely open.

4. Scan the barcode on the FilmArray pouch using the barcode scanner. Pouch identification (Lot Number and Serial Number), Pouch Type and Protocol are preprogrammed in the rectangular barcode located on the FilmArray pouch. The information will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, Pouch Type and Protocol can be manually entered from the information provided on the pouch label into the appropriate fields. **The barcode cannot be scanned prior to placing the pouch in the instrument. A "Cannot scan now" message will be displayed.**
5. Enter the Sample ID. The Sample ID can be entered manually or scanned in by using the barcode scanner when a barcoded Sample ID (accession label) is used.
6. If necessary, select a protocol from the Protocol drop down list.
7. Enter a user name and password in the Name and Password fields.
8. Close the FilmArray Instrument lid.
9. Click 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. **The bead-beater apparatus can be heard as a high-pitched noise (whine) during the first minute of operation.**
10. When the run is finished, follow the on-screen instructions to open the instrument and remove the pouch. Wear gloves when removing the pouch. Examine the pouch for any drips to verify that the pouch did not leak during testing.
11. Immediately discard the pouch in a biohazard container.
12. Results are automatically displayed in the report section of the screen. The run file is automatically saved in the FilmArray database and the report can be printed and/or saved as a PDF file.

10.0 Interpretation of Results

When 2nd stage PCR is complete, the FilmArray Instrument performs a high resolution DNA melting analysis on the PCR products and measures the fluorescence signal generated in each well. The FilmArray Software then performs several analyses and assigns a final assay result.

10.1 Analysis of Melting Curves

The FilmArray Software evaluates the DNA melt curve for each well of the 2nd stage PCR array to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature (Tm) of the curve. The Tm value is then compared against the expected Tm range for the assay. If the software determines that the melt curve is positive and the Tm falls inside the assay-specific Tm range, the melt curve is called positive. If the software determines that the melt curve is negative or is not in the appropriate Tm range, the melt curve is called negative.

10.2 Analysis of Replicates

Once melt curves have been identified, the software evaluates the three replicates for each assay to determine the assay result. For an assay to be called positive, at least two of the three associated melt curves must be called positive, and the Tm for at least two of the three positive melt curves must be similar (within 1°C). Assays that do not meet these criteria are called negative.

10.3 Organism Interpretation

Interpretations for many of the organisms are based on the results of a single assay. Interpretations for *Haemophilus influenzae* and the *Staphylococcus*, *Streptococcus,* and *Enterobacteriaceae* groups rely on the results of several assays. The antimicrobial resistance genes are also based on the result of a single assay; however, the test results are only reported when specific organisms are also detected in the same sample. This section provides an explanation of the test results and guidelines for actions to be taken based on the test result. This section also contains information about known assay limitations (e.g., cross-reactivity,

strains that are not detected) that may be important in the interpretation of the test result and in correlating the FilmArray results with the result of standard culture and biochemical identification.

10.3.1 Gram-Positive Bacteria

Enterococcus

The assay detects the major species associated with *Enterococcus* bloodstream infections (*E. faecium* and *E. faecalis*) as well as several less common species of varying clinical relevance including: *E. avium, E. casseliflavus, E. dispar, E. durans, E. gallinarum, and E. hirae. E. raffinosus,* which is occasionally isolated from clinical specimens, will not be detected by the BCID Panel.

Limited cross-reactivity with coagulase-negative staphylococci has been observed. Sequence analysis and empirical testing suggest that this cross-reactivity may occur when select species (*S. haemolyticus*, *S. epidermidis*, and *S. capitis*) are in the blood culture at a very high concentration.

Listeria monocytogenes

There are 12 known serovars of *L. monocytogenes*, however; only three serovars (1/2a, 1/2b and 4b) account for more than 90% of human cases of listeriosis. The BCID Panel detects all known serovars. Sequence analysis predicts that cross-reactivity with some strains of atypical *Listeria innocua* is possible.

Staphylococcus

The BCID Panel contains three assays for the detection of *Staphylococcus* species. The *Staphylococcus aureus* assay and two multi-species assays (Staphylococcus1 and Staphylococcus2). The Saureus assay detects all strains of *S. aureus* and does not cross-react with other organisms, including other species of *Staphylococcus*. The multi-species assays detect the most prevalent coagulase-negative *Staphylococcus* (CoNS) species encountered in blood culture specimens and can also react with high levels of *S. aureus*.

The FilmArray Software integrates the results of the three *Staphylococcus* assays into a final *Staphylococcus* test result. If all three assays are negative, the test result will be *Staphylococcus* Not Detected. If any of the three assays is positive, the result will be *Staphylococcus* Detected. Results for the Saureus assay (positive or negative) determine the *Staphylococcus aureus* test result (Detected or Not Detected, respectively).

The two multi-species assays (Staphlyococcus1 and Staphylococcus2) detect the most commonly encountered CoNS recovered for positive blood cultures. However, this is a large and diverse group and detection by BCID Panel assays is variable. The following species are expected to be reliably detected by the BCID Panel at organism concentrations observed in positive blood cultures (~ 5 x 10^6 CFU/mL): *S. aureus, S. caprae, S. cohnii, S. epidermidis, S. haemolyticus, S. hominis, S. lugdunensis,* and *S. xylosus*. The following species are detected with reduced sensitivity and are likely to be detected at higher concentrations (~ 1 x 10^7 to 10^8 CFU/mL) but may not always be detected in positive blood cultures: *S. capitis, S. pasteuri, S. pettenkoferi, S. saprophyticus, S. saccharolyticus, S. warneri*. The following species are unlikely to be detected by the BCID Panel due to sequence mismatches with the BCID Panel assays: *S. auricularis, S. carnosus, S. lentus, S. schleiferi* ssp. *schleiferi, S. sciuri,* Non-*aureus* coagulase-positive species (*S. intermedius, S. pseudointermedius, and S. schleiferi* ssp. *coagulans*).

Streptococcus

The BCID Panel contains four assays for the detection of *Streptococcus* species. Species-specific assays are included for the detection of Group A Strep, Group B Strep, and *S. pneumoniae*. The fourth assay is a multi-species assay (Streptococcus) designed to react with select Viridans group and other *Streptococcus* species encountered in blood culture specimens.

Streptococcus agalactiae, *Streptococcus pneumoniae*, *Streptococcus pyogenes* are three of the most important streptococci to identify from blood cultures and each is detected by the BCID Panel with a species-specific assay. The gene target for each assay is either found only in the

species of interest or is highly conserved within the species of interest. No cross-reactivity with other streptococci is predicted for these assays.

The multi-species Streptococcus assay reacts with the following species of Lancefield groups A – H at levels observed in positive blood cultures: *S. anginosus*, *S. bovis, S. constellatus*, *S. dysgalactiae*, *S. equinis, S. gallolyticus, S. gordonii*, *S. intermedius*, *S. mitis, S. mutans*, *S. oralis, S. parasanguinis*, S. *pseudopneumoniae*, *S. salivarius,* and *S. sanguinis.* Streptococci that are not listed here are rare and have not been tested. The Streptococcus assay may demonstrate variable to no reactivity with those species.

10.3.2 Gram-Negative Bacteria

Acinetobacter baumannii

Acinetobacter baumannii is part of the *Acinetobacter calcoaceticus-baumannii* (ACB) complex. In addition to *A. baumannii*, the complex includes *A. calcoaceticus, A. pittii*, and *A. nosocomialis*. These species are genetically and phenotypically related and cannot be reliably differentiated from each other using current microbial identification methods. The BCID Panel assay detects *A. baumannii*; however, it also detects some strains of the non-*baumannii* species with varying sensitivity. Discrepancies between the BCID Panel test result and microbial identification may be caused by misidentification of non-*baumannii* members of the ACB complex as *A. baumannii*. No cross-reactivity with other *Acinetobacter* species outside of the ACB complex is expected.

Haemophilus influenzae

The BCID Panel contains two different assays for the detection of *H. influenzae*. If either or both of the assays are positive, the test result will be *Haemophilus influenzae* Detected. If both the Hinfluenzae1 and Hinfluenzae2 assays are negative, the test result will be *Haemophilus influenzae* Not Detected.

Neisseria meningitidis

The BCID Panel detects encapsulated strains of *N. meningitidis*. Unencapsulated strains are generally considered non-virulent species of the normal nasopharyngeal flora and are not detected by the BCID Panel.

Pseudomonas aeruginosa

The assay detects *P. aeruginosa* and does not cross-react with other *Pseudomonas* species or closely-related bacteria.

Enterobacteriaceae

The BCID Panel includes seven assays to detect members of the *Enterobacteriaceae* family. Six genus/species specific assays are included for the detection of *Enterobacter cloacae* (and other *E. cloacae* complex species); *Escherichia coli, Klebsiella oxytoca*, *Klebsiella pneumoniae, Proteus* spp*.,* and *Serratia marcescens*. A seventh assay (the Enteric assay) will react with some (not all) species detected by the other six assays; however, its primary function is to detect other less common, but clinically relevant members of the *Enterobacteriaceae* family. A positive result for any of the seven *Enterobacteriaceae* associated assays will generate an *Enterobacteriaceae* Detected result. Each specific genus/species assay result will also be reported independently. Results for the Enteric assay are not reported independently, but are incorporated into the *Enterobacteriaceae* test result. Negative results for all seven assays will generate an *Enterobacteriaceae* Not Detected result.

Enterobacter cloacae complex

The *Enterobacter cloacae* complex is comprised of six species (*E. asburiae*, *E. cloacae*, *E. hormaechei*, *E. kobei*, *E. ludwigii*, and *E. nimipressuralis*) that may all be identified as *E. cloacae* by phenotypic laboratory methods. Of the six complex species, the BCID Panel assay detects *E. cloacae* (subspecies *cloacae* and *dissolvens*), *E. asburiae*, and *E. hormaechei*. Detection of *E. kobei, E. ludwigii*, and *E. nimipressuralis* is not expected and the clinical significance of these species is uncertain. Cross-reactivity with the closely related *Enterobacter cancerogenus* (which has previously been described as a member of the *E. cloacae* complex; also known as *E.*

taylorae) is possible. Cross-reactivity with *Enterobacter aerogenes* and two former *Enterobacter* (*Cronobacter sakazakii* and *Pantoea agglomerans*) has not been observed.

Escherichia coli

The BCID Panel 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 potentially emerging pathogen.

Klebsiella oxytoca

The BCID Panel assay does not cross-react with other *Klebsiella* or *Enterobacteriaceae* 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. Similarly, a few variant strains of *K. oxytoca* have been identified that will not be detected as *K. oxytoca* by the BCID Panel; however, these variants are detected by the Enteric assay and reported as *Enterobacteriaceae* Detected.

Klebsiella pneumoniae

The BCID Panel assay detects *K. pneumoniae* (including three subspecies; ssp. *pneumoniae*, ssp. *ozaenae*, and ssp. *rhinosclermatis*) and *K. variicola. K. variicola* is a closely related species to *K. pneumoniae* that has been isolated from clinical specimens. The assay does not cross-react with *Klebsiella oxytoca*. However, the closely related *Raoultella* (formerly *Klebsiella*) *ornithinolytica* can be misidentified as *K. oxytoca* and exhibits cross-reactivity with the assay.

Proteus

The BCID Panel assay detects four of five characterized species within the genus (*P. mirabilis*, *P. hauseri*, *P. penneri*, and *P. vulagaris*). *P. mirabilis*, *P. penneri*, and *P. vulagaris* are considered opportunistic human pathogens, with *P. mirabilis* being the most common. The fifth species in the genus, *P. myxofaciens*, is not a known human pathogen and detection is not expected.

Serratia marcescens

Serratia marcescens is the primary human pathogen within the *Serratia* genus, though rare cases of human infection with other *Serratia* species (*S. plymuthica, S. liquefaciens*, *S. rubidaea*, *S. odorifera, S. ficaria* and *S. fonticola*) have been described. The BCID Panel assay was designed to detect *S. marcescens*, but will exhibit variable reactivity with select *Serratia* species as well. Based on sequence analysis and empirical testing, *S. ficaria* and *S. entomophila* (non-human pathogen) can be reliably detected. Reactivity with *S. odorifera* and *S. rubidaea* (rare human pathogens) is also possible, depending on the amount of organism in the specimen. *S. liquefaciens, S. plymuthica, S. fonticola*, *S. grimesii,* and *S. proteamaculans* will not be detected. In addition to *Serratia* species, cross-reactivity has also been observed between the assay and a specific strain of *Pseudomonas aeruginosa* (ATCC 25619), the soil bacterium *Pseudomonas putida*, and *Raoultella ornithinolytica* (commonly misidentified as *Klebsiella oxytoca*).

Other *Enterobacteriaceae*

If any of the species specific assays described above are positive, the *Enterobacteriaceae* result will be Detected. However, the *Enterobacteriaceae* family includes many additional species that are not covered by the species specific assays. The BCID Enteric assay detects many, but not all species of *Enterobacteriaceae*, including *Cedeceae davisiae*, *Citrobacter* spp., *Cronobacter (Enterobacter) sakazakii*, *Enterobacter* spp. *(including E. aerogenes*), *Escherichia* spp., *Kluyvera ascorbata*, *Leclercia adecarboxylata*, *Raoultella* spp., *Salmonella* spp., and *Yokenella regensburgei*. The Enteric assay will not detect *Morganella* spp., *Providencia* spp., *Rahnella* spp., or most *Yersinia* spp.

Based on sequence data, the Enteric assay may react with some other *Enterobacteriaceae* when present at high levels (> $1x10^8$ CFU/mL) including *Edwardsiella tarda*, *Hafnia alvei*, *Pantoea* spp., some *Serratia* spp., *Tatumella ptyseos*, and *Yersinia enterocolitica*. The Enteric assay may also react with members of *Enterobacteriaceae* family that are not human pathogens (*Brenneria* spp., *Dickeya* spp., *Erwinia* spp., *Pectobacterium* spp., etc.).

10.3.3 Yeast

Candida albicans, Candida glabrata, Candida krusei, Candida parapsilosis, and Candida tropicalis

Species-specific assays are included in the BCID Panel for each of the five most common *Candida* species associated with candidemia (*Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis*). Based on *in silico* analysis and empirical testing, each assay is specific for detection of the indicated species with the following exceptions:

- *Candida albicans* is closely related to *Candida dubliniensis* and misidentification of these species by laboratory methods does occur. Cross-reactivity between the Calbicans assay and *C. dubliniensis* has not been observed but sequence analysis predicts that cross-reactivity with *C. dubliniensis* is possible.
- The BCID Panel assay for detection of *C. parapsilosis* cross-reacts with *Candida orthopsilosis*. Prior to being designated as unique species, *C. orthopsilosis* and *C. metapsilosis* were classified as Group II and Group III *Candida parapsilosis,* respectively. Both are closely related to *Candida parapsilosis* and can be misidentified as *Candida parapsilosis* using standard identification methods. The BCID Panel assay for *C. parapsilosis* will not detect *C. metapsilosis*; however, amplification of *C. orthopsilosis* is predicted by sequence analysis and has been confirmed. *In silico* analysis suggests that cross-reactivity with *C. multigemmis* may also be possible, though this has not been observed.

NOTE: *Candida krusei* is also known as *Issatchenkia orientalis* and *Pichia kudriavzevkii*, therefore reactivity with isolates identified as these species does not represent cross-reactivity.

10.3.4 Antimicrobial Resistance Genes Interpretation

The test results for antimicrobial resistance genes are only reported when an associated organism is detected in the same test (see table below).

Resistance Genes	Associated Organism
mecA	*Staphylococcus*
vanA/B*	*Enterococcus*
KPC	Any *Enterobacteriaceae, A. baumannii,* and/or *P. aeruginosa*

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

Detected – when an appropriate organism is detected and the antimicrobial resistance gene assay is positive.

Not Detected – when an appropriate organism is detected and the antimicrobial resistance gene assay is negative.

N/A – when NO appropriate organism is detected regardless of the result for the antimicrobial resistance gene assay.

11.0 Reporting

11.1 FilmArray BCID Test Report

The FilmArray BCID test report is automatically displayed upon completion of a run and contains three sections, the Run Summary, the Results Summary, and the Run Details. The test report can be saved as a PDF or printed.

11.1.1 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 tests were negative then None will be displayed in the Detected field. Antimicrobial resistance genes with a result of Detected or Not Detected will be listed in the corresponding field of the summary. Controls are listed as Passed, Failed or Invalid. See the Controls Field section below for detailed information about the interpretation of controls and appropriate follow-up in the case of control failures.

11.1.2 Result Summary

The **Results Summary – Interpretations** section of the test report lists the result for each target tested by the panel. Possible results for each organism are Detected, Not Detected, or Invalid. Possible results for antimicrobial resistance genes are Detected, Not Detected, N/A, or Invalid. See Results Summary section below for detailed information about interpretation of test results and appropriate follow-up for Invalid results.

11.1.3 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, Instrument Communication Error, or Software Error), the protocol that was used to perform the test, the identity of the operator that performed the test, and the instrument used to perform the test.

Once a run has completed, it is possible to edit the Sample ID. If this information has been changed, an additional section called **Change History** will be added to the test report. This Change History section lists the field that was changed, the original entry, the revised entry, the operator that made the change, and the date that the change was made. Sample ID is the only field of the report that can be changed.

11.2 Result Reporting in LIS

11.2.1 Positive Result(s)

Enter **Positive by FilmArray PCR for:** and the name of the organism, along with any resistance genes, detected.

The following comments should be added to the report to describe all of the pathogens that are included in the panel:

The following organisms were tested for by FilmArray PCR:
Acinetobacter baumannii, Candida albicans, Candida glabrata, Candida krusei, Candida parapsilosis, Candida tropicalis, Enterococcus, Enterobacteriaceae, Enterbacter cloacae* complex, *Escherichia coli, Haemophilus influenzae, Klebsiella oxytoca, Klebsiella pneumoniae, Listeria monocytogenes, Neisseria meningitidis, Proteus, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus, Staphylococcus aureus , Streptococcus, Streptococcus agalacticae* (Group B), *Streptococcus pneumoniae,* and *Streptococcus pyogenes* (Group A)*.

If applicable, add the following comment to describe all of the resistance genes included in the panel:

The following resistance genes were tested for by FilmArray PCR:
Van A/B **(vancomycin-resistance genes for *Enterococcus*),** ***mecA*** **(methicillin-resistance gene for *Staphylococcus*), and KPC (carbapenem-resistance gene for any *Enterobacteriaceae, Acinetobacter baumannii,* and, *Pseudomonas aeruginosa*)**

If the FilmArray BCID Test Report indicates that the resistance gene test is not applicable (N/A), this comment does not need to be added.

11.2.2 Negative Result

Enter **Negative by FilmArray PCR for all organisms tested.** The following comment should be added to the report to describe all of the pathogens that are included in the panel:

The following organisms were tested for by FilmArray PCR:
Acinetobacter baumannii, Candida albicans, Candida glabrata, Candida krusei, Candida parapsilosis, Candida tropicalis, Enterococcus, Enterobacteriaceae, Enterbacter cloacae* complex, *Escherichia coli, Haemophilus influenzae, Klebsiella oxytoca, Klebsiella pneumoniae, Listeria monocytogenes, Neisseria meningitidis, Proteus, Pseudomonas

***aeruginosa, Serratia marcescens, Staphylococcus, Staphylococcus aureus , Streptococcus, Streptococcus agalacticae* (Group B), *Streptococcus pneumoniae,* and *Streptococcus pyogenes* (Group A).**

12.0 Quality Control & Quality Assurance

12.1 Process Controls

Two process controls are included in each pouch:

- **DNA Process Control**

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

- **PCR2 Control**

  The PCR2 Control assay detects a DNA target that is dried into the wells of the array along with the corresponding primers. A positive result indicates that 2nd stage PCR was successful.

Both control assays must be positive for the test run to pass. When either control fails, the Controls field of the test report (upper right hand corner) will display Failed and all results will be listed as Invalid. If the controls fail, the sample should be retested using a new pouch.

12.2 External Controls

External control materials are prepared in-house by pooling suspensions of ATCC control strains or previously characterized clinical isolates. The organisms are separated into two sets. The table below lists the targets in each set and the expected results when tested on BCID assay.

Table 1 - Blood Culture ID Panel Controls

Target	Strain	BC Multi 1	BC Multi 2
Acinetobacter baumannii	ATCC 19606	Negative	Positive
Candida albicans	ATCC 90028	Positive	Negative
Candida glabrata	ATCC 15126	Positive	Negative
Candida krusei	ATCC 14243	Positive	Negative
Candida parapsilosis	ATCC 22019	Positive	Negative
Candida tropicalis	ATCC 750	Positive	Negative
Enterobacter cloacae	ATCC 13047	Negative	Positive
Enterococcus	ATCC 51299	Positive	Negative
Escherichia coli	ATCC 25922	Negative	Positive
Haemophilus influenzae	ATCC 35056	Negative	Positive
Klebsiella oxytoca	Clinical strain	Negative	Positive
Klebsiella pneumoniae	ATCC BAA-1705	Negative	Positive
Listeria monocytogenes	Clinical strain	Positive	Negative
Neisseria meningitidis	N/A	Negative	Negative
Proteus	ATCC 35659	Negative	Positive
Pseudomonas aeruginosa	ATCC 27853	Negative	Positive
Serratia marcescens	ATCC 8100	Negative	Positive
Staphylococcus aureus	ATCC 43300	Positive	Negative
Streptococcus agalacticae	ATCC 12386	Positive	Negative
Streptococcus pneumoniae	ATCC 49619	Positive	Negative
Streptococcus pyogenes	ATCC 19615	Positive	Negative

External control materials must be used to evaluate each new lot or shipment of FilmArray Respiratory Panels. External controls must be tested every 30 d while a lot is in use. Quality control results should be entered into the LIS. Notify technical specialist or supervisor if results are not as expected, and do not report any patient results until the issue has been resolved. Repeat testing using new external controls.

12.3 Report Review

All test results entered into LIS should be reviewed by a second technologist on the same shift or the beginning of the next shift. Results should be compared to the printed results from the BioFire computer. The review should be documented on the BioFire Report with date and initials.

12.4 Environmental Surveillance Testing

Due to the nature and sensitivity of nucleic acid amplification testing, practices are necessary to safeguard against contamination of laboratory equipment and surfaces used to process and perform the PCR assays. Environmental contamination with organisms, target nucleic acids, or amplicons may lead to contamination of patient samples that could result in false-positive test results. Improper decontamination can lead to false-negative test results.

1. Surfaces to be Tested
   The biosafety cabinet work surfaces and the pouch loading station should be sampled and tested at least once per month or following a potential issue with contamination. More surfaces may be tested following a potential contamination event. The instrument and surrounding surfaces may be tested if a leaking pouch is discovered.
2. Sample Collection and Preparation
   Use one tube with 0.5 mL of sterile saline and one swab to sample both the biosafety cabinet and the pouch loading station. Dip the swab into the saline and swab the areas by rotating the swab 2 to 3 times across the surfaces (~ 10 cm^2). After sampling the surface areas, place the swab into the saline and break off the upper portion of the shaft. Cap the tube and briefly vortex the sample.
3. Sample Testing
   Load and test one BCID Panel following the procedure described above.
4. Interpretation of Test Results
   A negative assay result indicates that no nucleic acids for any of the targets were detected. A positive assay result indicates that target material was detected, and environmental contamination has occurred. An unresolved assay result indicates the presence of an inhibitory substance or internal control amplicon.
5. Decontamination
   Surfaces that test positive for any of the targets should be decontaminated. Surface samples that yield unresolved results should also be decontaminated and cleaned. If an environmental surface tests positive for any of the targets, additional surveillance testing should be performed after decontamination to verify that decontamination was successful. Consult with the director or technical specialist.

13.0 Limitations

1. The performance of this test has only been evaluated by the manufacturer with the BD BACTEC Plus Aerobic/F Plus blood culture bottle. In-house verification testing was performed with the Bact/ALERT SA (aerobic) and SN (anaerobic) bottles.
2. This product should not be used to test blood culture media that contain charcoal. Charcoal containing media may contain non-viable organisms and/or nucleic acid at levels that can be detected by the FilmArray BCID Panel.
3. Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for the FilmArray antimicrobial resistance gene assays does not indicate antimicrobial susceptibility. Subculturing and standard susceptibility testing of isolates is required to determine antimicrobial susceptibility.

4. The results for the FilmArray antimicrobial resistance gene assays do not specifically link the resistance gene to the associated organism. In mixed growth, the FilmArray BCID Panel does not specifically attribute *vanA/B*mediated vancomycin resistance to a specific *Enterococcus* sp.; *mecA*-mediated methicillin resistance to either *S. aureus* or other *Staphylococcus* sp.*;* or KPC mediated carbapenem-resistance to *Enterobacteriaceae, Acinetobacter baumannii* or *Pseudomonas aeruginosa*.
5. The FilmArray BCID Panel does not contain assays for obligate anaerobic organisms that might be recovered in blood culture.
6. Resin beads contained in blood culture media have been shown to cause pouch control failures and affect assay performance. Blood culture samples must be collected in a manner that prevents resin beads from being introduced into the sample and FilmArray test. Needles with a larger bore size (e.g., 18-gauge) should not be used to obtain blood culture samples if the medium contains resin beads. Use a 28-gauge needle to remove the blood culture sample without removing resin beads.
7. Blood culture samples must be tested within 8 h of being flagged as positive by a continuously monitoring blood culture instrument.
8. This test has not been validated for testing samples other than positive blood culture samples that demonstrate the presence of organisms by Gram stain evaluation.
9. Results from this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
10. The FilmArray BCID Panel does not detect all species in the *Enterobacteriaceae* family. *Morganella, Rahnella*, *Providencia* spp., and most *Yersinia* spp. will not be detected.
11. The FilmArray BCID Panel does not detect all species of *Enterococcus, Proteus, Staphylococcus* or *Streptococcus*.
12. The FilmArray BCID Enterococcus assay may cross-react with high level of coagulase-negative staphylococci (CoNS). Cross-reactivity may occur when select species of CoNS (*S. haemolyticus*, *S. epidermidis*, and *S. capitis*) are in the blood culture at high concentrations.
13. Discrepancies between the BCID Panel test result and microbial identification may be caused by the inability to reliability differentiate species based on standard microbial identification methods. Examples include *Acinetobacter baumannii*, *Klebsiella oxytoca* and *Raoultella ornithinolytica*.
14. 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 negative values resulting from improperly collected, transported, or handled samples.
15. A negative FilmArray BCID 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. Negative results should not be used as the sole basis for diagnosis, treatment, or other management decisions.
16. Organism and amplicon contamination may produce erroneous results for this test. Particular attention should be given to the Laboratory Precautions noted under the Warnings and Precautions section.
17. 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.
18. If three or more distinct organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.
19. Cross-reactivity with organisms other than those listed in the Organism Interpretation section above may lead to erroneous results.
20. The effect of interfering substances has only been evaluated for those listed in the labeling. Interference by substances other than those described in the Interference section below could lead to erroneous results.

14.0 Verification Information

The BioFire Blood Culture ID Panel Assay has been cleared by the FDA for clinical diagnostic testing. No modifications have been made to the FDA-cleared assay. The manufacturer's published findings for analytical sensitivity and specificity can be found in the package insert. In this evaluation, ATCC strains and clinical isolates were used to verify the accuracy and precision of the assay.

Assay Accuracy

The materials used to evaluate the performance of the FilmArray BCID included samples of positive patient blood cultures in BacT/ALERT SA and SN bottles and seeded blood culture samples. To maximize the evaluation of panel targets, samples were pooled together to create one test sample with multiple targets. Patient samples were tested within 8 h of the bottle being flagged as positive by the instrument, or frozen at -70°C for later testing. Frozen samples were thawed at room temperature and mixed prior to testing. Seeded samples were created by harvesting colonies from agar plates to prepare a 1 McFarland suspension that could be pooled together in a blood culture matrix. Isolates used for seeding samples included both clinical and ATCC strains.

A total of 19 pooled samples were tested. These 19 samples contained a total of 120 panel targets. Overall, the assay demonstrated 98% sensitivity and 100% specificity. No false-positive results were encountered. Table 2 below summarizes the results of the organisms detected. The two isolates that were not detected by the assay included one Viridans strep and one *Morganella morganii.* Both of these represent assay limitations rather than detection failures.

Other organisms that were included in the pooled test samples that do not contain targets detected by the BCID assay included 1 *Bacillus* spp., 2 *Corynebacterium* spp., 1 *Fusobacterium* spp., 1 *Pichia kluyneri/fermentans*, and 2 *Stenotrophomonas maltophilia* isolates. None of these organisms reacted with the assay. The *Pichia* had previously been identified as a *C. krusei* by the BD Phoenix system. When the isolate did not react on the BCID Panel it was sent to ARUP Laboratories and confirmed to be *Pichia kluyneri/fermentans* by DNA sequencing.

All of the resistance gene targets included in the test samples were detected by the assay. One "empty cassette" *S. aureus* isolate that was PCR-positive with the BD MAX MRSA PCR assay was included. This isolate did not produce a false-positive result with the BCID Panel as was seen with the BD MAX assay.

Table 2 – Targets tested and detected by the BCID Panel

Table 2 Organism	Clinical Targets Tested	ATCC Targets Tested	Patient Blood Cx Tested	Seeded Blood Cx Tested	Number Detected	% Correct	Target Negative Tested	% Correct
Acinetobacter baumannii	4	1	1	4	5	100	23	100
Candida albicans	3	1	1	3	4	100	24	100
Candida glabrata	3	1	3	1	4	100	24	100
Candida krusei	3	1	0	4	4	100	24	100
Candida parapsilosis	3	1	0	4	4	100	24	100
Candida tropicalis	3	1	1	3	4	100	24	100
Enterobacter cloacae	3	1	0	4	4	100	24	100
Enterobacteriaceae	3	0	1	2	2	67	1	100
Enterococcus spp.	6	1	3	4	7	100	21	100
vanA/B - positive	3	1	1	3	4	100	24	100
vanA/B - negative	3	0	2	1	3	100	25	100
Escherichia coli	3	1	3	1	4	100	24	100
Haemophilus influenzae	4	1	0	5	5	100	23	100
Klebsiella oxytoca	3	0	1	2	3	100	25	100
Klebsiella pneumoniae	5	1	3	3	6	100	22	100
KPC - positive	2	1	0	3	3	100	25	100
KPC - negative	3	0	3	0	3	100	25	100
Listeria monocytogenes	5	0	0	5	5	100	23	100
Neisseria meningitidis	3	1	0	4	4	100	24	100
Proteus spp.	3	1	2	2	4	100	24	100
Pseudomonas aeruginosa	4	1	4	1	5	100	23	100
Serratia marcescens	3	1	0	4	4	100	24	100
Staphylococcus aureus	5	1	4	2	6	100	22	100
mecA - positive	2	1	2	1	3	100	25	100
mecA - negative	3	0	2	1	3	100	25	100
Staphylococcus (Coag-neg)	4	1	3	2	5	100	23	100
mecA - positive	2	0	2	0	2	100	26	100
mecA - negative	2	1	2	1	3	100	25	100
Streptococcus spp.	3	0	3	0	2	67	25	100
Streptococcus agalactiae	4	1	0	5	5	100	23	100
Streptococcus pneumoniae	5	1	2	4	6	100	22	100
Streptococcus pyogenes	3	1	3	1	4	100	24	100
Organism	Clinical Targets Tested	ATCC Targets Tested	Patient Blood Cx Tested	Seeded Blood Cx Tested	Number Detected	% Correct	Target Negative Tested	% Correct
Total	97	23	43	77	118	98%	740	100%

Precision

Precision was evaluated with 3 pooled specimens. These specimens were created by preparing a suspension of each organism equivalent to a 1.0 McFarland turbidity standard using sterile saline. Sample pools were prepared by mixing aliquots of the organism suspensions with 11 mL of a negative patient blood culture. Each sample was tested in 4 replicates. A total of 8 different users performed the testing. Each sample test produced the expected results. The pooling scheme is outlined below in Table 3.

Table 3 – Pooled samples used for precision testing.

Organism	Strain	Organism Volume	Blood Culture Volume	Approx. Final Vol.
Pool 1				
Candida albicans	90028	0.1 mL	11 mL	12 mL
Candida krusei	14243	0.1 mL		
Streptococcus agalactiae	12386	0.1 mL		
Neisseria meningitidis	13077	0.1 mL		
Pseudomonas aeruginosa	27853	0.1 mL		
Staphylococcus aureus (MRSA)	43300	0.1 mL		
Streptococcus pyogenes	19615	0.1 mL		
Pool 2				
Enterococcus faecalis	51299	0.2 mL	11 mL	12 mL
Staphylococcus epidermidis (MSSE)	12228	0.4 mL		
Acinetobacter baumannii	19606	0.1 mL		
Candida glabrata	15126	0.1 mL		
Candida tropicalis	750	0.2 mL		
Enterobacter cloacae	13047	0.3 mL		
Klebsiella oxytoca	Clinical	0.1 mL		
Listeria monocytogenes	Clinical	0.1 mL		
Escherichia coli	25922	0.1 mL		
Pool 3				
Candida parapsilosis	22019	0.1 mL	11 mL	12 mL
Klebsiella pneumoniae	BAA-1705	0.1 mL		
Proteus mirabilis	35659	0.1 mL		
Serratia marcescens	8100	0.1 mL		
Haemophilus influenzae	35056	0.1 mL		
Streptococcus pneumoniae	49619	0.1 mL		

15.0 References

1. FilmArray® Blood Culture Identification (BCID) Panel Instruction Booklet, RFIT-PRT-0101 June 2013.