BIOFIRE® FILMARRAY® Pneumonia Panel

Rx Only

Instructions for Use	www.biofiredx.com/e-labeling/ITI0075
Quick Guide	www.biofiredx.com/e-labeling/ITI0080
Safety Data Sheet (SDS)	www.biofiredx.com/e-labeling/ITI0085
Pouch Module	www.biofiredx.com/e-labeling/ITIFA20PNEUMO20

CE 2797

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Customer and Technical Support Information *For more information on how to contact Customer and Technical Support, refer to Appendix A.	**U.S. Customers**	Phone: 1-800-735-6544 (toll free) E-mail: BioFireSupport@biomerieux.com Website: www.biofiredx.com
	Outside of the U.S.	Contact the local bioMérieux sales representative or an authorized distributor

INTENDED PURPOSE

Intended Use

The BIOFIRE® FILMARRAY® Pneumonia Panel (BIOFIRE Pneumonia Panel) is a multiplexed nucleic acid test intended for use with BIOFIRE® FILMARRAY® 2.0 (BIOFIRE 2.0) or BIOFIRE® FILMARRAY® TORCH (BIOFIRE TORCH) systems for the simultaneous detection and identification of multiple respiratory viral and bacterial nucleic acids, as well as select antimicrobial resistance genes, in sputum-like specimens (induced or expectorated sputum, or endotracheal aspirates) or bronchoalveolar lavage (BAL)-like specimens (BAL or mini-BAL) obtained from individuals suspected of lower respiratory tract infection.

The following bacteria are reported semi-quantitatively with bins representing approximately 10^4, 10^5, 10^6, or ≥10^7 genomic copies of bacterial nucleic acid per milliliter (copies/mL) of specimen, to aid in estimating relative abundance of nucleic acid from these common bacteria within a specimen:

Bacteria reported with bins of 10^4, 10^5, 10^6, or ≥10^7 copies/mL		
Acinetobacter calcoaceticus-baumannii complex	*Klebsiella oxytoca*	*Serratia marcescens*
Enterobacter cloacae complex	*Klebsiella pneumoniae* group	*Staphylococcus aureus*
Escherichia coli	*Moraxella catarrhalis*	*Streptococcus agalactiae*
Haemophilus influenzae	*Proteus* spp.	*Streptococcus pneumoniae*
Klebsiella aerogenes	*Pseudomonas aeruginosa*	*Streptococcus pyogenes*

The following atypical bacteria, viruses, and antimicrobial resistance genes are reported qualitatively:

Atypical Bacteria		
Chlamydia pneumoniae	*Legionella pneumophila*	*Mycoplasma pneumoniae*
Viruses		
Adenovirus	Human rhinovirus/enterovirus	Parainfluenza virus
Coronavirus	Influenza A virus	Respiratory syncytial virus
Human metapneumovirus	Influenza B virus	

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

The detection and identification of specific viral and bacterial nucleic acids, as well as the estimation of relative abundance of nucleic acid from common bacterial analytes, within specimens collected from individuals exhibiting signs and/or symptoms of a respiratory infection, aids in the diagnosis of lower respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, pathogens below the limit of detection, or in the case of bacterial analytes, present at levels below the lowest reported 10^4 copies/mL bin. Detection of analytes does not rule out co-infection with other organisms; the agent(s) detected by the BIOFIRE Pneumonia Panel may not be the definite cause of disease. Additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible lower respiratory tract infection.

Detection of bacterial nucleic acid may be indicative of colonizing or normal respiratory flora and may not indicate the causative agent of pneumonia. Semi-quantitative Bin (copies/mL) results generated by the BIOFIRE Pneumonia Panel are not equivalent to CFU/mL and do not consistently correlate with the quantity of bacterial analytes compared to CFU/mL. For specimens with multiple bacteria detected, the relative abundance of nucleic acids (copies/mL) may not correlate with the relative abundance of bacteria as determined by culture (CFU/mL). Clinical correlation is advised to determine significance of semi-quantitative Bin (copies/mL) for clinical management.

The antimicrobial resistance gene detected may or may not be associated with the agent(s) responsible for disease. Negative results for these antimicrobial resistance gene assays do not indicate susceptibility to corresponding classes of antimicrobials, as multiple mechanisms of antimicrobial resistance exist.

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 Pneumonia Panel results should be used in conjunction with culture results for determination of bacterial susceptibility or resistance.

Due to the genetic similarity between human rhinovirus and enterovirus, the test cannot reliably differentiate them. A positive Rhinovirus/Enterovirus result should be followed up using an alternate method (***e.g.***, cell culture or sequence analysis) if differentiation is required.

Culture is required to identify pathogens not detected by the BIOFIRE Pneumonia Panel, to further speciate analytes in genus, complex, or group results if desired, to identify bacterial pathogens present below the 10^4 copies/mL bin if desired, and for antimicrobial susceptibility testing.

Intended User and Use Environment

The BIOFIRE Pneumonia Panel is intended for use by trained medical and laboratory professionals in a laboratory setting or under the supervision of a trained laboratory professional.

SUMMARY AND EXPLANATION OF THE TEST

Pathogens infecting the lower respiratory tract cause acute local and systemic disease, with the most severe cases occurring in children, the elderly, and immunocompromised individuals. Lower respiratory symptoms can include shortness of breath, weakness, high fever, coughing, and fatigue. Due to the similarity of diseases caused by many viruses and bacteria, diagnosis based on clinical symptoms alone is difficult. Identification of potential causative agents, as well as the relative abundance of common bacterial agents, provides data to aid the physician in determining appropriate patient treatment and public health response for disease containment. The BIOFIRE Pneumonia Panel is designed for simultaneous detection and identification of the pathogens of lower respiratory tract infection and associated antimicrobial resistance (AMR) genes described below, as well as estimated relative abundance of nucleic acid from the common bacterial agents listed.

Summary of Detected Organisms

Bacteria (reported semi-quantitatively with copies/mL bin results)

***Acinetobacter calcoaceticus-baumannii* complex** – *Acinetobacter baumannii* is a ubiquitous, non-fermentative, gram-negative coccobacillus that primarily acts as an opportunistic pathogen infecting critically-ill patients. It is an uncommon member of the normal skin flora. Hospital-acquired pneumonia is the most common infection caused by *A. baumannii*, although other nosocomial infections caused by *A. baumannii* are increasing in frequency.[1] Several related *Acinetobacter* species cannot be reliably differentiated from *A. baumannii* by some manual or automated phenotypic microbial identification systems. These species, which include *A. calcoaceticus, A. pittii* (genomospecies 3), *A. seifertii*, and *A. nosocomialis* (genomospecies 13TU) are grouped together with *A. baumannii*, into the ***Acinetobacter calcoaceticus-baumannii* (ACB) complex**. Multi-drug resistant strains demonstrate resistance to most antibiotic classes, including carbapenems. Various carbapenem-hydrolyzing metallo-β-lactamases may be carried by these bacteria.[2]

***Enterobacter cloacae* complex (ECC)** – *Enterobacter cloacae* and associated members of the *E. cloacae* complex (*Enterobacter asburiae, Enterobacter hormaechei* (and subspecies)*, Enterobacter kobei, Enterobacter ludwigii*, *Enterobacter mori*, and *Enterobacter roggenkampii*) are gram-negative rod shaped bacteria belonging to the *Enterobacteriaceae* family. Members of the complex are generally identified as '*E. cloacae*' by standard methods, though the group is genetically heterogeneous and descriptions of the complex members vary between analysis methods. Additional genetically similar *Enterobacter* species and subspecies (*Enterobacter bugandensis*, *Enterobacter cancerogenus*, *Enterobacter chengduensis*, *Enterobacter soli*, and *Enterobacter hormaechei ssp. xiangfangensis* (also described as *E. xiangfangensis*), among others) have been discussed in the context of the ECC or recently identified as potential new ECC members by comprehensive whole genome evaluations, but there is inconsistency in the literature as to which species should be considered species within the complex.[3–8] The *E. cloacae* complex has been implicated in numerous nosocomial infections, which are notable for their severity in ICU patients.[9] *E. cloacae*, *E. hormaechei* and *E. asburiae* are the ECC species most frequently implicated in pneumonia, with *E. cloacae* being found to carry more types of β-lactamases than the other species.[10] Up to 31% of nosocomial *Enterobacter* pneumonia cases in the ICU are associated with strains demonstrating cephalosporin resistance.[11]

Escherichia coli is an enteric gram-negative bacterium that is part of the normal flora of the intestines of humans and animals. *E. coli* is found in approximately 6-9% of community-acquired pneumonia (CAP) and hospital-acquired pneumonia (HAP) and is responsible for 1.2% of all pneumonia diagnoses in the U.S.[12,13] *E. coli* acts as an opportunistic causal agent of pneumonia and the prognosis associated with *E. coli*-caused pneumonia is poorer than that for pneumonia caused by other bacteria and viruses.[13] As with other *Enterobacteriaceae*, extended spectrum β-lactamases (ESBLs) pose a significant antibiotic resistance problem.

Haemophilus influenzae is a gram-negative coccobacillus, isolated exclusively from humans[14], that can be present as normal flora of the oropharynx and can cause infections when introduced into the lower respiratory tract.[15–17] Strains of *H. influenzae* are divided into two groups based on the presence or absence of a capsular polysaccharide.[18,19] 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.[18,19] 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[18], with a mortality rate of 13-20%.[19] Approximately 20-35% of isolated strains are resistant to amoxicillin.[18] No evidence of carriage of the antimicrobial resistance genes detected by the BIOFIRE Pneumonia Panel has been recorded in the literature.[20,21]

Klebsiella aerogenes (formerly *Enterobacter aerogenes*[22]) is a gram-negative rod-shaped bacterium found as a member of the normal gut flora. In recent years, *K. aerogenes* has become the third leading cause of nosocomial pneumonias, after *Escherichia coli* and *Pseudomonas aeruginosa*.[23] It is speculated that this relatively recent emergence is due to the overuse of extended-spectrum cephalosporins; *Enterobacter* strains (including *K. aerogenes*) isolated from patients in Europe and Israel have shown high resistance to β-lactam antibiotics.[24]

Klebsiella oxytoca is an aerobic gram-negative, rod-shaped bacterium carried on mucosal surfaces (nasopharynx and bowel) and found in agricultural environments. Opportunistic infections due to *K. oxytoca* include soft tissue infections, urinary tract infections, pneumonia, and septicemia. While *K. oxytoca* is a rare agent in community-acquired pneumonia, it is identified more frequently in life-threatening hospital-acquired pneumonia.[25] An increasing proportion of *K. oxytoca* bacteremia isolates demonstrate resistance to extended-spectrum β-lactams, especially when there is a history of prior antibiotic use[26]. Additionally, carbapenem resistance has been observed in nosocomial outbreaks of *K. oxytoca.*[27] 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.[28]

***Klebsiella pneumoniae* group** – The *Klebsiella pneumoniae* group includes three phylogroups, recently classified as distinct species; *K. pneumoniae* (KPI), *K. quasipneumoniae* (KPII), and *K. variicola* (KPIII).[29,30] All three species have many of the same virulence factors and share biochemical and genetic similarities, which makes it difficult to distinguish *K. quasipneumoniae* and *K. variicola* from *K. pneumoniae* clinically or by standard culture methods[31]. *Klebsiella pneumoniae* is a gram-negative rod-shaped bacterium found as part of the normal flora of the human mouth and skin.[29] However, when *K. pneumoniae* is aspirated into the lungs it can cause alveolar damage leading to pneumonia.[29] *K. pneumoniae* is associated most often with nosocomial infections in the elderly or immunocompromised.[32] *Klebsiella* spp. are opportunistic pathogens accounting for 7-14% of hospital-acquired pneumonia and ~8% of all nosocomial bacterial infections in the United States.[33] The mortality rate associated with *K. pneumoniae* infection of the lungs is in part due to the emergence of antibiotic resistance genes, such as carbapenmases, in these bacteria.[32,34]

Moraxella catarrhalis is an opportunistic gram-negative bacterial pathogen of the human respiratory tract. There is increasing recognition of the clinical relevance of this organism in lower respiratory tract infections of adults.[35] Only 1-3% of community-acquired pneumonia has been attributed to *M. catarrhalis*[36]; however, it is believed to be a significant pneumonia-causing pathogen for individuals who are elderly and/or malnourished, those who have underlying respiratory diseases such as COPD, and in cases of hospital-acquired pneumonia.[37,38] Mortality due to *M. catarrhalis* pneumonia is 10-29%, with the higher rate observed in patients with underlying respiratory disease and co-infections with other respiratory pathogens.[38] Most strains of *M. catarrhalis* now carry β -lactamases.[39]

Proteus* spp. –** Members of the gram-negative genus ***Proteus 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[40]; however, nosocomial outbreaks have also occurred.[41] Antimicrobial resistance has become an increasing problem in *Proteus* infections, with approximately 32% of isolates producing extended-spectrum β-lactamases.[42]

Pseudomonas aeruginosa is a gram-negative opportunistic pathogen that is a leading cause of nosocomial infections and is responsible for 10% of all hospital-acquired infections.[43] It is has been reported as the causative agent in both community-acquired pneumonia (CAP) and hospital-acquired pneumonia (HAP), often associated with ventilator use. *P. aeruginosa* is susceptible to a limited number of antibiotics (antipseudomonal penicillins and cephalosporins, carbapenems, and fluoroquinolones)[44], and multi-drug resistant (MDR) *P. aeruginosa* infection is becoming an increasing problem in hospitals.[43] The carbapenemase, KPC, has been identified in isolates of *P. aeruginosa.*[45]

Serratia marcescens – *Serratia* are gram-negative bacteria that are common nosocomial pathogens and colonizers. *S. marcescens* is the primary pathogenic species of the *Serratia* genus. It is of particular concern due to its emerging antibiotic resistance to commonly used agents like β-lactams, aminoglycosides, carbapenems, and fluoroquinolones. Non-pigmented *S. marcescens* are more resistant to antibiotics and are associated with most outbreaks.[46] Transmission may occur from person to person contact, via medical apparatus, intravenous fluids, or other solutions.[47]

Staphylococcus aureus is a gram-positive coccus that grows in grape-like clusters. A common, opportunistic bacterium, *S. aureus* is capable of causing a wide range of diseases and is considered the most clinically important human pathogen in the *Staphylococcus* genus. *S. aureus* possesses extensive virulence factors, has various strategies to evade the host immune response, and has become resistant to many therapeutic agents.[48] It is among the most common etiologic agents in lower respiratory tract infections comprising 3-14% of all cases of community-acquired pneumonia (CAP) and is, at 17%, the most frequently reported isolate in hospital-acquired pneumonia (HAP).[49] It is estimated that approximately 40% of *S. aureus* isolates may be methicillin resistant.[50] The primary mediator of methicillin resistance in staphylococci is acquisition of the *mecA* gene.

Streptococcus agalactiae (Group B *Streptococcus* or GBS) are gram-positive, catalase-negative cocci that grow in chains or pairs. *Streptococcus* species are frequently found as commensal bacteria on mucous membranes, and are occasionally present as transient skin microbiota.[48] Streptococci have historically been grouped as β-hemolytic or non-β-hemolytic, pyogenic (pus-forming) or non-pyogenic, 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 β-hemolytic.[48] *S. agalacticae* 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.[48] In adult patients, the spectrum of *S. agalacticae* infections includes bacteremia, pneumonia, meningitis, and endocarditis.[48]

Streptococcus pneumoniae is a gram-positive bacterium that colonizes the upper respiratory tract and is the most frequently isolated respiratory pathogen in community-acquired pneumonia. *S. pneumoniae* was responsible for approximately 30,400 invasive infections in the U.S. in 2016, leading to an estimated 3,690 deaths.[51] There are two licensed multivalent pneumococcal vaccines in the US (PPV23 and PCV13) which are recommended for neonates, immunocompromised, and those over the age of 65, and help reduce the risk of both invasive disease and pneumococcal pneumonia by 50-80%.[52]

Streptococcus pyogenes (Group A *Streptococcus* or GAS) colonizes the human skin and upper respiratory tract, with these sites serving as primary focal sites of infections and principal reservoirs of transmission of this gram-positive bacterium.[48] *S. pyogenes* possesses complex virulence mechanisms to avoid host defenses[53,54] and is responsible for deep or invasive infections, especially bacteremia, sepsis, and deep soft tissue infections.[48] More recently, *S. pyogenes* has been identified as a rare causal agent of pneumonia especially in the elderly or others with underlying health problems.[55] Interestingly, the peak season for *S. pyogenes* infections seems to coincide with the peak season for influenza virus and patients infected with both have a higher mortality rate.[55]

Atypical Bacteria

Chlamydia pneumoniae (previously known as *Chlamydophila pneumoniae*) is an obligate intracellular bacterium that causes acute respiratory infections and is a common cause of community-acquired atypical (walking) pneumonia and bronchitis.[56–58] *C. pneumoniae* has an incubation period of approximately three weeks and can be transmitted from asymptomatic carriers.[58] Outbreaks occur in schools, military barracks, and nursing homes.[59] No peak season has been identified for *C. pneumoniae* infections.

Legionella pneumophila is a gram-negative rod with fastidious nutritional requirements such as dependence on L- cysteine and iron[60], and is the causative agent of Legionnaires' Disease. Aqueous and soil environments are assumed to be the natural reservoirs of many different types of *Legionella* species.[60] About 90% of human legionellosis cases reported worldwide have been attributed to *L. pneumophila*.[60] Approximately 5% of hospitalized adult pneumonia cases have been attributed to *Legionella* species.[60] Clinical features associated with Legionnaires' disease include fever, body aches, and cough, sometimes accompanied by shortness of breath, headache, confusion, nausea, or diarrhea.[61]

Mycoplasma pneumoniae is another bacterial agent of community-acquired atypical pneumonia, occurring frequently in outbreak situations.[62,63] Incubation time for *M. pneumoniae* infection is approximately 1 to 4 weeks.[64] *M. pneumoniae* respiratory disease does not have a defined season of highest incidence but epidemics have a periodicity of 3-7 years.[63]

Antimicrobial Resistance Genes

CTX-M (Extended spectrum β-lactamase (ESBL)) - CTX-M is a class A extended-spectrum β-lactamase that originated from a mobilization of chromosomal genes (*bla*) from *Kluyvera* spp. and confers resistance to a broad spectrum of cephalosporins. This group of β-lactamases can be plasmid-borne and the *bla*$_{CTX-M}$ gene may be found in multiple copies per cell within a variety of gram-negative hosts. Phylogenetic analyses of CTX-M describes five main lineages or phylogroups (CTX-M groups 1, 2, 8, 9, and 25) and over 150 types or variants.[65] CTX-M ESBLs are predominantly found in the *Enterobacteriaceae* family. However, they have also been reported in other non-enteric gram-negative bacteria such as *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Acinetobacter baumannii, Vibrio* spp. and *Aeromonas* spp.[66] Over the last decade, CTX-M enzymes have overtaken other ESBLs, including TEM and SHV ESBL variants, in prevalence.[67]

IMP (Carbapenem resistance) - IMP (Imipenem) β-lactamases are plasmid-borne metallo-β-lactamases (MBLs) belonging to Ambler class B1 MBLs. Many distinct IMP types have been identified (numbered 1-60) which have the potential to confer different levels of antibiotic resistance to broad-spectrum β-lactams like carbapenems, cephamycins, and oxymino cephalosporins.[68,69] MBLs hydrolyze almost all β-lactams, rendering ineffective products, resulting in bacterial resistance to this class of antibiotics.[70] Because conventional guidelines recommend β-lactams as a preferred treatment for patients with community-acquired pneumonia (CAP), the increased development of MBL resistance in lower respiratory pathogens is of particular concern.[71] Carriage of a *bla*$_{IMP}$ gene has been detected in strains of *Serratia marcescens, Klebsiella pneumoniae, Pseudomonas, Escherichia coli,* and *Enterobacter cloacae.*[72]

KPC (Carbapenem resistance) - The *Klebsiella pneumoniae* carbapenemase gene (*bla*$_{KPC}$ or referred to here as KPC), confers resistance to the carbapenem class of β-lactams and currently is thought to be the most common and rapidly emerging carbapenemase in the United States. Though originally isolated from *Klebsiella pneumonia*e, the gene has since disseminated to other genera/species including *Acinetobacter*, *Pseudomonas*, *Enterobacter, Serratia, Salmonella, Escherichia coli, Klebsiella oxytoca*, and other *Enterobacteriaceae*. As of late 2016 there are several known KPC variants that have been identified (named up to KPC-26). The most commonly isolated types are KPC-2 and KPC-3.[73] 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).[73]

***mecA/C* and MREJ (MRSA) (Methicillin resistance)** - Methicillin-resistant (MR) staphylococci are a serious 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 (e.g. oxacillin/methicillin).[48] The primary mechanism of methicillin resistance is through acquisition of the *mecA* gene that encodes a penicillin binding protein (PBP2a) that has low affinity for β-lactams. The *mecA* gene is carried on a chromosomally-integrated mobile genetic element called the staphylococcal cassette chromosome *mec* (*SCCmec*). In 2011, an *SCCmec* type XI cassette carrying a divergent *mecA* homologue (*mecC*), which also confers methicillin resistance, was identified in Europe.[74] In *S. aureus*, the mec cassette integrates into a specific region in the *S. aureus* genome[75,76], this insertion creates MREJ (SCCmec right-extremity junction). The junction, or point of insertion of *mecA/C* in the cassette, can vary leading to a variety of MREJ types (i-xx). The BIOFIRE Pneumonia Panel MREJ assay is designed to detect this specific integration event in the *S. aureus* genome.

NDM (Carbapenem resistance) - The New Delhi metallo-β-lactamase (NDM) is a plasmid-mediated enzyme that confers resistance to all current β-lactam antibiotics, with the exception of aztreonam.[77,78] There are 16 different NDM types that may be found in a variety of gram-negative species, with NDM-1 recognized throughout the world. The *bla*$_{NDM}$ gene is widely and rapidly disseminated throughout the *Enterobacteriaceae*, as well as other gram-negative bacteria.[78–82] The plasmids encoding NDM are easily transferable and capable of wide rearrangement, suggestive of extensive transmission, as well as plasticity, amongst bacterial populations[79]. Multi-drug resistant NDM-producing bacteria are now the most prevalent carbapenemase producers in Europe, and this trend is expected to continue worldwide.

OXA-48-like (Carbapenem resistance) - The oxacillinase (OXA) β-lactamases are a group of primarily plasmid-mediated enzymes that confer resistance to penicillins, cephalosporins, and carbapenems. The *bla*$_{OXA-48}$ gene, and several OXA-48-like variants have been identified in various gram-negative bacteria in the *Enterobacteriaceae* family.[83,84] OXA-48 hydrolyzes penicillins at a high level, carbapenems at a low level with greater activity against imipenem than meropenem[83], and demonstrates very weak activity against expanded-spectrum cephalosporins.[84] The BIOFIRE Pneumonia Panel OXA-48-like assay targets OXA-48, as well as the -162[85], -181[86], -199[87], -204[88], -232[89], -244[90], -245[90], -252[91], -370[92], -484[93], and -505[94] variants in the OXA-48 family. Each of these variants have from one to five amino acid substitutions, but maintain the hydrolytic properties and substrate profile of OXA-48.[84,90,92] Other variants that retain activity against extended-spectrum cephalosporins but do not have carbapenemase activity (OXA-163, -247, -405, -436, -438, and -439), are not targeted by the assay.

VIM (Carbapenem resistance) - Verona Integron-Encoded Metallo-β-Lactamase (VIM) are integron-encoded carbapenemases. There are reports of both plasmid and chromosomal localization of the *bla*$_{VIM}$ integron[95]; however, the majority of *bla*$_{VIM}$ alleles are found on plasmids. There are approximately distinct 50 types of VIM types. VIMs are found mainly in gram-negative bacteria, including enteric bacteria, with a vast majority associated with various species of genus *Pseudomonas*.

Viruses

Adenoviruses (AdV) are a diverse group of non-enveloped DNA viruses with seven species (A to G).[96] Adenovirus species B, C, and E cause acute respiratory disease, but all types have been associated with human disease.[97] Adenoviruses (species A, D, F, and G) can cause a variety of illnesses, including cystitis, gastroenteritis, and conjunctivitis.[98] Outbreaks often occur in institutional settings such as military training, long-term care facilities, and pediatric tertiary-care hospitals, due to high rates of transmission in closed populations.[99–101] Adenoviruses are shed for long periods of time and persist on surfaces in an infective state.[101]

Coronaviruses (CoV) - Human coronaviruses were established as respiratory pathogens in the 1960s. Four predominant serological variants (229E, OC43, HKU1, NL63) associated with human disease are detected by the BIOFIRE Pneumonia Panel and reported together as Coronavirus. These viruses are most commonly associated with upper respiratory tract infections; however, they have also been detected in individuals with lower respiratory tract infections.[102–104] Coronaviruses have been associated with croup and exacerbation of asthma.[102,105] Coronavirus infection occurs more often in the winter and there appears to be a periodicity of epidemics for some strains.[103] Coronavirus infections (with the exception of SARS and MERS-CoV) are generally self-limiting.

Human metapneumovirus (hMPV) is in the family Paramyxoviridae.[106] HMPV was discovered in 2001 as a respiratory pathogen in children.[107] Further studies confirmed hMPV infections in persons of all ages.[108] The two genotypes, A and B, can circulate at the same time and do not appear to differ in the severity of illness.[106] HMPV is the second leading cause of bronchiolitis in young children.[106] Additionally, infection can result in a broad range of upper and lower respiratory symptoms: cough, rhinorrhea, wheeze, dyspnea, and fever.[109] HMPV is estimated to be responsible for 5-7% of respiratory tract infections in children and 3% among individuals of all ages.[109] The seasonal peak of hMPV is winter and early spring and often co-occurs with the seasonal peak of respiratory syncytial virus (RSV).[110]

Human rhinoviruses (HRV) and **enteroviruses (EV)** are related RNA viruses in the *Picornaviridae* family.[111] Both viruses contain the same viral RNA genome organization and analogous secondary structures making them difficult to distinguish genetically. There are more than 100 serotypes of human rhinovirus based on the serology of the capsid protein.[111] Rhinovirus is noted as causing the “common cold”, but may also be involved in precipitating asthma attacks and severe complications.[111] Enteroviruses are divided into four species that include a total of at least 89 distinct types. Individual types can be associated with different clinical manifestations, including nonspecific respiratory illnesses in infants or adults.[112] Both rhinoviruses and enterovirus are prevalent year round.[113,114]

Influenza A virus and Influenza B virus (Flu A/Flu B) are RNA viruses in the *Orthomyxoviridae* family. During annual influenza epidemics, 5-20% of the population is affected with upper respiratory tract infections with rapid onset of fever.[115] The dominant type of influenza virus varies often due to antigenic drift and shift.[116] Influenza A virus can be subtyped by the hemagglutinin (H) and neuraminidase (N) genes; influenza A virus subtypes H1N1 and H3N2 are the strains that most

commonly infect humans. More severe disease and increased mortality are associated with H3N2 subtype.[116] During the 2009-10 influenza season, influenza A virus (H1N1)pdm09 became the dominant circulating influenza virus, accounting for approximately 99% of reported influenza infections and has since replaced pre-2009 H1N1 strains.[117] Currently, at least four antiviral medications are available for influenza treatment – amantadine, rimantadine, zanamivir, and oseltamivir – with type-specific efficacy and drug resistance arising with the spread of new strains of the virus.[118] Complications with viral or bacterial pneumonia increase mortality from influenza infections.[119]

Parainfluenza viruses (PIVs) are RNA viruses in the *Paramyxoviridae* family. In the 1950s, parainfluenza viruses were determined to be respiratory pathogens different from influenza viruses.[120] Parainfluenza viruses are divided into four types (1-4) that are detected by the BIOFIRE Pneumonia Panel and reported together as Parainfluenza Virus. Parainfluenza virus 1 causes biennial epidemics in the fall, with 50% of croup cases attributed to this virus.[120] Parainfluenza virus 2 causes epidemics every one to two years, which may alternate with parainfluenza virus 1 circulation.[120] Children less than six months old are particularly susceptible to parainfluenza virus 3 infection, with outbreaks occurring in neonatal intensive care units. PIV3 is associated with the highest mortality and morbidity of all strains[121] and epidemics are most common in the spring and summer.[120] Parainfluenza virus 4 infection affects all age groups but because of infrequent detection periodicity of infection has not been established.[122,123]

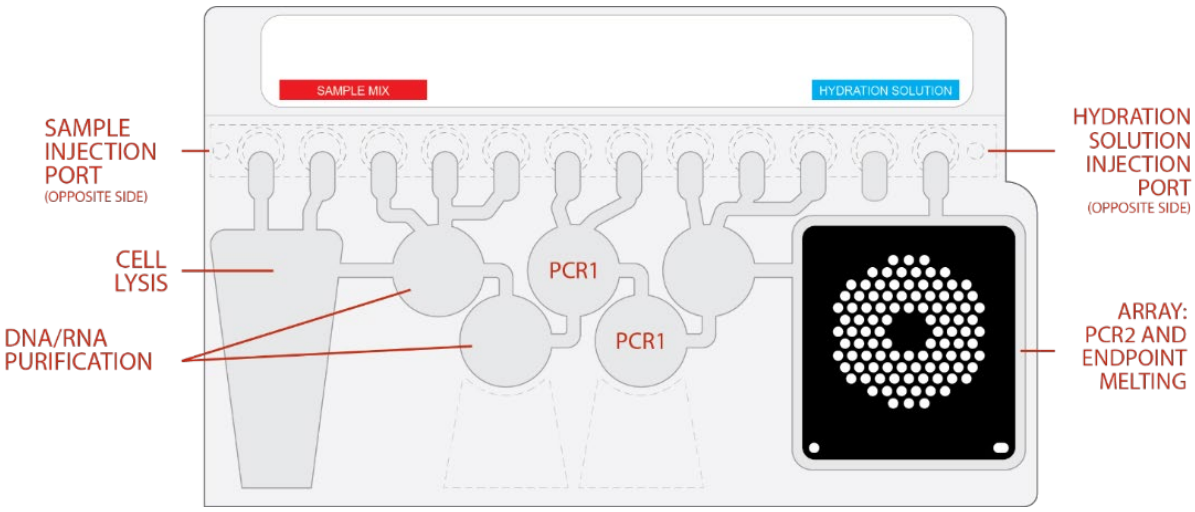
Respiratory syncytial virus (RSV) is a member of the RNA viruses in the *Paramyxoviridae* family, related to human metapneumoviruses and parainfluenza viruses.[124] RSV has two major subtypes (A and B), which vary annually in their prevalence.[125] RSV is the most common cause of severe respiratory disease in infants, with acute bronchiolitis as the major cause of hospitalization.[124] RSV is now also recognized as an important pathogen in adults, although adult infections are in general less severe and limited to the upper respiratory tract.[126] Peak activity of RSV is typically in January and February.[127]

PRINCIPLE OF THE PROCEDURE

The BIOFIRE Pneumonia Panel pouch is a closed system disposable that stores all the necessary reagents for sample preparation, reverse transcription, polymerase chain reaction (PCR), and detection in order to isolate, amplify, and detect nucleic acid from multiple lower respiratory pathogens within a single bronchoalveolar lavage (BAL)-like (BAL or mini-BAL) or sputum-like (sputum or ETA) specimen. After sample collection, the user injects hydration solution and sample combined with Sample Buffer into the pouch, places the pouch into a BIOFIRE® FILMARRAY® System Instrument module, and starts a run. The entire run process takes about one 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).
- Extracts and purifies all nucleic acids from the sample using magnetic bead technology.
- Performs nested multiplex PCR by:
    - First performing reverse transcription and a single, large volume, massively-multiplexed reaction (PCR1), and
    - 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 Pneumonia Panel array.
- For the BIOFIRE Pneumonia Panel, the system also uses real-time amplification data from the assays relative to a Quantified Standard Material (QSM) included in the pouch to provide an estimated value in genomic copies per milliliter (copies/mL) for bacterial analytes.



MATERIALS PROVIDED

Each kit contains sufficient reagents to test 30 samples (30-test kit; RFIT-ASY-0144):

- Individually-packaged BIOFIRE Pneumonia Panel pouches
- Single-use Sample Buffer ampoules
- Single-use pre-filled Hydration Injection Vials (blue)
- Single-use Sample Injection Vials (red)
- Individually-packaged Sample Swabs
- BIOFIRE Pneumonia Panel Pouch Module Software

  This software is required to run the BIOFIRE Pneumonia Panel Pouch and can be downloaded at www.biofiredx.com/e-labeling/ITIFA20PNEUMO20 if not already installed on the BIOFIRE 2.0 or BIOFIRE TORCH Systems

MATERIALS REQUIRED BUT NOT PROVIDED

- BIOFIRE System including:
  - BIOFIRE 2.0 or BIOFIRE TORCH Systems
    - including accompanying system-specific core software and panel-specific pouch module software
  - BIOFIRE® Pouch Loading Station
- 10% bleach solution or a similar disinfectant

WARNINGS AND PRECAUTIONS

General Precautions

1. For *in vitro* diagnostic use only.
2. A trained healthcare professional should carefully interpret the results from the BIOFIRE Pneumonia Panel in conjunction with a patient's signs and symptoms, results from other diagnostic tests, and any relevant epidemiological information.
3. BIOFIRE Pneumonia Panel pouches are only for use with BIOFIRE 2.0, and BIOFIRE TORCH systems.
4. Always check the expiration date on the pouch. Do not use a pouch after its expiration date.
5. BIOFIRE pouches are stored under vacuum in individually-wrapped canisters. To preserve the integrity of the pouch vacuum for proper operation, be sure that an instrument module will be available and operational before unwrapping any pouches for loading.

Safety Precautions

1. Wear appropriate Personal Protective Equipment (PPE), including (but not limited to) disposable clean powder-free gloves and lab coats. Protect skin, eyes, and mucus membranes. Change gloves often when handling reagents or samples.
2. Handle all samples and waste materials as if they were capable of transmitting infectious agents. Observe safety guidelines such as those outlined in:
    - CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories.*[128]
    - CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections*[129]
3. Follow your institution's safety procedures for handling biological samples.
4. If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to a state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
5. Dispose of materials used in this assay, including reagents, samples, and used buffer vials, according to federal, state, and local regulations.
6. Sample Buffer contains Guanidinium chloride and Triton X100.

    The following statements apply.

    - Health Hazards
        - Acute Toxicity, oral (Category 4)
            - H302 – Harmful if swallowed.
        - Skin corrosion/irritation (Category 2)
            - H315 - Causes skin irritation.
        - Serious eye damage/eye irritation (Category 1)
            - H318 - Causes serious eye damage.
    - Environment Hazards
        - Hazardous to the aquatic environment, acute aquatic hazard (Category 1)

        - H400 - Very toxic to aquatic life.
    - Hazardous to the aquatic environment, long-term aquatic hazard (Category 1)
        - H410 - Very toxic to aquatic life with long lasting effects.
- Precautionary Statements
    - Prevention
        - P273 – Avoid release to the environment.
        - P280 – Wear protective gloves/protective clothing/eye protections/face protection.
- Response
        - P332 + P313 - If skin irritation occurs: Get medical advice/attention.
        - P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
        - P301 + P312 - IF SWALLOWED: Call a POISON CENTRE/doctor if you feel unwell.
        - P337 + P313 - If eye irritation persists: Get medical advice/attention.

Please refer to the BIOFIRE Pneumonia Panel Safety Data Sheet (SDS) for more information www.biofiredx.com/e-labeling/ITI0085.

7. Sample Buffer will form hazardous compounds and fumes when mixed with bleach or other disinfectants.

WARNING: Never add Bleach to Sample Buffer or sample waste.

8. Bleach, a recommended disinfectant, is corrosive and may cause severe irritation or damage to eyes and skin. Vapor or mist may irritate the respiratory tract. Bleach is harmful if swallowed or inhaled.
    - Eye contact: Hold eye open and rinse with water for 15-20 minutes. Remove contact lenses after the first 5 minutes and continue rinsing eye. Seek medical attention.
    - Skin contact: Immediately flush skin with plenty of water for at least 15 minutes. If irritation develops, seek medical attention.
    - Ingestion: Do not induce vomiting. Drink a glassful of water. If irritation develops, seek medical attention.
    - Please refer to the appropriate Safety Data Sheet (SDS) for more information.

Laboratory Precautions

1. Preventing organism contamination

Due to the sensitive nature of the BIOFIRE Pneumonia Panel, it is important to guard against contamination of the sample and work area by carefully following the testing process outlined in this instruction document, including these guidelines:

- Laboratory personnel may carry or shed common respiratory pathogens asymptomatically and can inadvertently contaminate the specimen while it is being processed. To avoid this, handle specimens in a biosafety cabinet. If a biosafety cabinet is not used, a dead air box (e.g., AirClean PCR workstation), a splash shield (e.g., Bel-Art Scienceware Splash Shields), or a face shield should be used when preparing specimens for testing.
- Laboratory personnel with active respiratory symptoms (runny nose, cough) should wear a standard surgical mask (or equivalent) and should avoid touching the mask while handling specimens.
- Do not handle specimens or pouches in a biosafety cabinet which is used for pathogen culture or immunofluorescence testing.

- Prior to processing specimens, thoroughly clean both the work area and the Pouch Loading Station using a suitable cleaner such as freshly prepared 10% bleach or a similar disinfectant. To avoid residue build-up and potential damage to the specimen or interference from disinfectants, wipe disinfected surfaces with water.
- Specimens and pouches should be handled and/or tested one-at-a-time. Always change gloves and clean the work area between each pouch and specimen.
- Use clean gloves when removing Sample Buffer ampoules and Sample/Hydration Injection Vials from bulk packaging bags, and reseal bulk packaging bags when not in use.
- Avoid collecting or handling specimens in areas that are exposed to vaccine material for pathogens included on the BIOFIRE Pneumonia Panel (*e.g.* influenza). Particular care should be taken during these processes to avoid contamination.

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 BIOFIRE Pneumonia 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, in addition to those above, to prevent amplicon contamination:

- Discard used pouches in a biohazard container immediately after the run has completed.
- Avoid excessive handling of pouches after test runs.
- Change gloves after handling a used pouch.
- Avoid exposing pouches to sharp edges or anything that might cause a puncture.

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 appropriate BIOFIRE Operator's Manual. DO NOT PERFORM ADDITIONAL TESTING UNTIL THE AREA HAS BEEN DECONTAMINATED.

Precaution Related to Public Health Reporting

Local, state, and national/federal regulations for notification of reportable disease are continually updated and include a number of organisms and antimicrobial resistance for surveillance and outbreak investigations.

Additionally, the Centers for Disease Control and Prevention (CDC) recommends that when pathogens from reportable diseases are detected by a culture independent diagnostic test (CIDT), the laboratory should facilitate obtaining the isolate or clinical materials for submission to the appropriate public health laboratory to aid in outbreak detection and epidemiological investigations.

Laboratories are responsible for following the applicable regulations and reporting requirements in their region and should consult public health authorities for isolate and/or clinical sample submission guidelines.

Precaution Related REACH Regulation (EC 1907/2006)

This statement only applies to countries within the European Union (EU) with regard to the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation (EC 1907/2006):

It is recommended that all material associated with the test, including the material used to clean up spills, contaminated packaging, and/or unused and expired IVD tests, is incinerated. Please ensure that you follow local regulations regarding disposal.

REAGENT STORAGE, HANDLING, AND STABILITY

1. Store the test kit, including reagent pouches and buffers, at room temperature (15–25 ºC).
2. Avoid storage of any materials near heating or cooling vents or in direct sunlight.
3. All kit components should be stored and used together. Do not use components from one kit with those of another kit. Discard any extra components from the kit after all pouches have been consumed.
4. 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 minutes).
5. Once a pouch has been loaded, the test run should be started as soon as possible (within approximately 60 minutes). Do not expose a loaded pouch to temperatures above 40°C (104°F) prior to testing.

SAMPLE REQUIREMENTS

The following table describes the requirements for specimen collection, preparation, and handling that will help ensure accurate test results. The detection of viral and bacterial nucleic acid (including AMR genes) is dependent upon proper specimen collection, handling, transportation, storage, and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results (false positive, false negative, or inaccurate bin results).

Specimen Type	**Bronchoalveolar lavage (BAL)-like specimens** • Including BAL and mini-BAL collected according to standard technique **Sputum-like specimens** • Including induced and expectorated sputum as well as endotracheal aspirate (ETA) collected according to standard technique
Minimum Sample Volume	Approximately 0.2 mL (200 µL) of specimen material will be captured by the Sample Swab for transfer into the test.
Transport and Storage	Specimens should be tested with the BIOFIRE Pneumonia Panel as soon as possible. If storage is required, specimens can be held: • Refrigerated for up to 1 day (2-8 °C)

NOTE: BAL-like or sputum-like specimens should <u>not</u> be centrifuged, pre-processed, treated with any mucolytic or decontaminating agents (e.g. MycoPrep, Sputasol, Snap n' Digest, DTT, sodium hydroxide, oxalic acid, trypsin, etc.), or placed into transport media before testing.

Note: In accordance with good laboratory practice recommendations, institutions should follow their own established rules for acceptance/rejection of sputum specimens (e.g. using Gram stain/Q-score) and therefore apply appropriate guidelines locally for acceptance/rejection of a sample for testing.

NOTE: Bleach can damage organisms/nucleic acids within the specimen, potentially causing false negative results. Contact between bleach and specimens during collection, disinfection, and testing procedures should be avoided.

PROCEDURE

Use clean gloves and other Personal Protective Equipment (PPE) when handling pouches and samples. Only prepare one BIOFIRE Pneumonia 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.

There is a risk of false positive results due to contamination of the specimen or testing area with organisms, their nucleic acids, or amplified product. Particular attention should be given to the Laboratory Precautions noted under the *Warnings and Precautions* section.

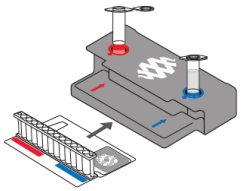
Refer to the appropriate BIOFIRE Operator's Manual for more details.

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.

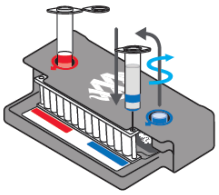
NOTE: The pouch may still be used even if the vacuum seal of the pouch is not intact. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.

3. 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.



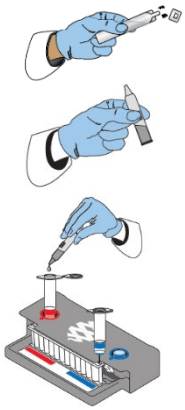
   - If the hydration solution is not automatically drawn into the pouch, repeat Step 2 to verify that the seal of the pouch hydration port was broken. If hydration solution is again not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.
5. Verify 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.

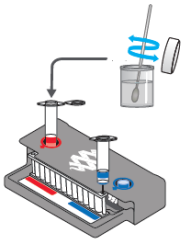
NOTE: Avoid touching the ampoule tip during handling, as this may introduce contamination.

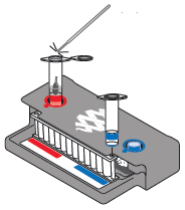


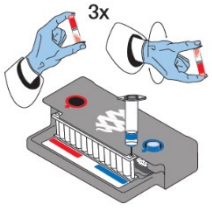
- To open the Sample Buffer ampoule:
    - If the ampoule has a plastic tab on the tip: Gently twist and remove tab at the tip of the Sample Buffer ampoule
    - If the ampoule does not have a plastic tab on the tip: Firmly pinch at textured plastic tab on the side of the ampoule until the seal snaps
- Invert the ampoule over the red-capped Sample Injection Vial and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.

NOTE: Avoid squeezing the ampoule additional times. This will generate foaming, which should be avoided.

WARNING: The Sample Buffer is harmful if swallowed and can cause serious eye damage and skin irritation.

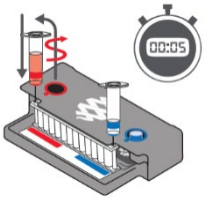






2. Using the Sample Swab provided in the test kit, thoroughly stir the BAL-like or sputum-like specimen for about 10 seconds.
3. Place the swab end of the Sample Swab into the Sample Injection Vial, then break off the swab handle.
4. Tightly close the lid of the Sample Injection Vial and discard the swab handle into the appropriate waste container.
5. Remove the Sample Injection Vial from the Pouch Loading Station and invert the vial at least 3 times to mix.
6. 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.

NOTE: Waiting 5 seconds decreases the risk of dripping and contamination from the sample.

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® FILMARRAY® 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 Operator's Manual for more detailed instructions.

BIOFIRE 2.0

1. Ensure that the BIOFIRE 2.0 system (instrument 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 an instrument, enter pouch, sample, and operator information.
3. Pouch identification (Lot Number and Serial Number) and Pouch Type 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, and Pouch Type 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 Pneumonia 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. Select and confirm the appropriate protocol from the Select Protocol dialog box. The BIOFIRE Pneumonia Panel uses two different protocols that should be selected according to the sample type (BAL or sputum) that is being tested.
6. Enter a user name and password in the Name and Password fields.

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

7. Review the entered run information on the screen. If correct, select Start Run.

    Once the run has started, the screen displays a list of the steps being performed by the instrument and the number of minutes remaining in the run.

NOTE: The bead-beater apparatus can be heard as a high-pitched noise during the first minute of operation.

8. When the run is finished, follow the on-screen instructions to remove the pouch, then immediately discard it in a biohazard waste container.
9. The run file is automatically saved in the BIOFIRE 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 (instrument) on the touch screen or scan the barcode on the BIOFIRE pouch using the barcode scanner.
3. Pouch identification (Lot Number and Serial Number) and Pouch Type 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, and Pouch Type 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 Pneumonia 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. Insert the pouch into the available Module (instrument).
    - Ensure that the pouch fitment label is lying flat on top of pouch and not folded over. As the pouch is inserted, the Module (instrument) will grab onto the pouch and pull it into the chamber.
6. Select and confirm the appropriate protocol from the Select Protocol dialog box. The BIOFIRE Pneumonia Panel uses two different protocols that should be selected according to the sample type (BAL or sputum) that is being tested.
7. Enter operator username and password, then select Next.

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

8. 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 Module (instrument) and the number of minutes remaining in the run.

NOTE: The bead-beater apparatus can be heard as a high-pitched noise during the first minute of operation.

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

QUALITY CONTROL

Process Controls

Two process controls are included in each pouch:

1. **RNA Process Control**

    The RNA Process Control assay targets an RNA 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 RNA Process Control result indicates that all steps carried out in the BIOFIRE Pneumonia Panel pouch were successful.

2. **Quantified Standard Material (QSM) Control**

   The QSM assay detects a quantified standard synthetic nucleic acid that is subject to all stages of the test process following sample lysis (bead beating). A positive QSM control result indicates that the expected level of QSM is present (approximately 10^6 copies/mL) for use in determining assay and bin results for bacterial analytes.

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 Software will automatically fail the run if the melting temperature (Tm) for either the RNA Process Control or the QSM is outside of an acceptable range (80.3-84.3°C for the RNA Process Control and 82.7-86.7°C for the QSM). 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 maintain records according to standard laboratory quality control practices.[130,131] Refer to the appropriate BIOFIRE Operator's Manual for instructions on obtaining control assay Tm values.

External Controls

External controls should be used in accordance with laboratory protocols and the appropriate accrediting organization requirements, as applicable. Molecular grade water or saline can be used as an external negative control. Previously characterized positive samples or negative samples spiked with well characterized organisms can be used as external positive controls. Commercially-produced control material may also be available from other manufacturers; use according to the control manufacturer's instructions.

INTERPRETATION OF RESULTS

Assay Interpretation

When PCR2 is complete, the instrument performs a DNA melting analysis on the PCR products and records the change in 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 curves. 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 of the curve is within the assay-specific Tm range, the melt curve is called positive. If the software determines that the Tm of the 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.

Analysis of assay results for bacteria. The assays in the BIOFIRE Pneumonia Panel for detection of bacteria that are reported semi-quantitatively are designed to amplify genes that are present in single copies within the chromosome of the target bacterium and are used to estimate genomic copies of bacterial nucleic acid per milliliter (copies/mL) of specimen. The BIOFIRE Software calculates an approximate value for each gene target based on real-time PCR amplification data relative to the QSM (internal reference of known quantity). Assays with no measurable amplification or a value below 10^3.5 copies/mL are called negative. Assays with a value equal to or greater than 10^3.5 copies/mL are called positive.

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, atypical bacteria, viruses, and antimicrobial resistance (AMR) genes as shown in Table 1. For most analytes detected by the BIOFIRE Pneumonia Panel, interpretations are based on the result of a single assay. However, results for *Staphylococcus aureus*, adenovirus, and the AMR genes require interpretation based on more than one assay result, as discussed in the relevant sections below.

Table 1. Analytes Detected by the BIOFIRE Pneumonia Panel

Bacteria		
Acinetobacter calcoaceticus-baumannii complex	*Klebsiella oxytoca*	*Serratia marcescens*
Enterobacter cloacae complex	*Klebsiella pneumoniae* group	*Staphylococcus aureus*
Escherichia coli	*Moraxella catarrhalis*	*Streptococcus agalactiae*
Haemophilus influenzae	*Proteus* spp.	*Streptococcus pneumoniae*
Klebsiella aerogenes	*Pseudomonas aeruginosa*	*Streptococcus pyogenes*
Atypical Bacteria		
Chlamydia pneumoniae	*Legionella pneumophila*	*Mycoplasma pneumoniae*
Viruses		
Adenovirus	Human rhinovirus/enterovirus	Parainfluenza virus
Coronavirus	Influenza A virus	Respiratory syncytial virus
Human metapneumovirus	Influenza B virus	
Antimicrobial Resistance Genes		
CTX-M	NDM	*mecA*/*C* and MREJ (MRSA)
IMP	OXA-48-like	
KPC	VIM	

Interpretations and Semi-quantitative Bin Results for Bacteria

The BIOFIRE Pneumonia Panel provides a Detected or Not Detected result as well as a semi-quantitative bin result (10^4 copies/mL, 10^5 copies/mL, 10^6 copies/mL, or ≥10^7 copies/mL) for most bacteria. The bin result represents the approximate number of specific bacterial genomes in the specimen and is intended to provide a simple assessment of relative abundance of nucleic acids from different bacteria in a lower respiratory specimen based on a molecular method.

For bacteria, negative assays (no measurable amplification or value less than 10^3.5 copies/mL) are reported as Not Detected. Positive assays are reported as Detected and a bin result is assigned based on the assay value. Each bin is defined by discrete upper and lower limits spanning a 1-log range of values (see Table 2) such that the bin result reflects the assay value within the nearest ±0.5-log.

Table 2. BIOFIRE Pneumonia Panel Bin Results for Bacteria

Assay Result		Reported Result and Bin Result	
Negative OR	<10^3.5 copies/mL	Not Detected	
Positive AND	≥10^3.5 – <10^4.5 copies/mL	Detected	10^4 copies/mL
Positive AND	≥10^4.5 – <10^5.5 copies/mL	Detected	10^5 copies/mL
Positive AND	≥10^5.5 – <10^6.5 copies/mL	Detected	10^6 copies/mL
Positive AND	≥10^6.5 copies/mL	Detected	≥10^7 copies/mL

Staphylococcus aureus

The BIOFIRE Pneumonia Panel pouch contains two different assays (Saureus1 and Saureus2) for the detection of *Staphylococcus aureus*. The BIOFIRE Software interprets each of these assays independently (as described above) and if one or a combination of the assays is positive, the result will be *Staphylococcus aureus* Detected with the appropriate bin result. If both assays are negative the result will be *Staphylococcus aureus* Not Detected.

NOTE: Detection of bacterial nucleic acid may be indicative of colonizing or normal respiratory flora and may not indicate the causative agent of pneumonia. Semi-quantitative Bin (copies/mL) results generated by the BIOFIRE Pneumonia Panel are not equivalent to CFU/mL and do not consistently correlate with the quantity of bacterial analytes compared to CFU/mL. For specimens with multiple bacteria detected, the relative abundance of nucleic acids (copies/mL) may not correlate with the relative abundance of bacteria as determined by culture (CFU/mL). Clinical correlation is advised to determine significance of semi-quantitative Bin (copies/mL) for clinical management.

Interpretations for Atypical Bacteria and Viruses

Results for most Atypical Bacteria and Viruses 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. However, Adenovirus detection is reported based on the results of multiple assays, as described below.

Adenovirus

The BIOFIRE Pneumonia Panel pouch contains three different assays (Adenovirus2, Adenovirus3, and Adenovirus7) for the detection of all species and serotypes of adenovirus. The BIOFIRE Software interprets each of these assays independently (as described above) and the results are combined as a final result for the virus. If one or any combination of assays is positive, the result will be Adenovirus Detected. If all assays are negative, the result will be Adenovirus Not Detected.

Interpretations for Antimicrobial Resistance (AMR) Genes

Results for AMR genes are also reported qualitatively (Detected/Not Detected) based on corresponding assays, but only if an applicable bacterium (i.e. potential carriers of the AMR gene; Table 3) is also detected (≥10^3.5 copies/mL) in the sample.

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

- 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).

Table 3. Antimicrobial Resistance (AMR) Genes and Applicable Organisms

AMR Gene Result	Applicable Bacteria
***mecA/C* and MREJ**	*Staphylococcus aureus*
CTX-M **IMP** **KPC** **NDM** **VIM**	*Acinetobacter calcoaceticus-baumannii* complex *Enterobacter cloacae* complex *Escherichia coli* *Klebsiella aerogenes* *Klebsiella oxytoca* *Klebsiella pneumoniae* group *Proteus* spp. *Pseudomonas aeruginosa* *Serratia marcescens*
OXA-48-like	*Enterobacter cloacae* complex *Escherichia coli* *Klebsiella aerogenes* *Klebsiella oxytoca* *Klebsiella pneumoniae* group *Proteus* spp. *Serratia marcescens*

Each AMR gene result is associated with a single corresponding assay except for the *mecA/C* and MREJ result, which is dependent on both the mecA/C assay and the MREJ assay (see Table 4). Detection of both *Staphylococcus aureus* and the mecA/C and MREJ markers is indicative of Methicillin Resistant *Staphylococcus aureus* (MRSA).

Table 4. Possible Assay Results and Interpretation for *mecA/C* and MREJ

BIOFIRE Pneumonia Panel Results		*Staphylococcus aureus*	mecA/C assay	MREJ Assay
Staphylococcus aureus mecA/C and MREJ	**Detected Detected**[a]	Detected	Positive	Positive
Staphylococcus aureus mecA/C and MREJ	**Detected Not Detected**	Detected	Positive	Negative
Staphylococcus aureus mecA/C and MREJ	**Detected Not Detected**	Detected	Negative	Positive
Staphylococcus aureus mecA/C and MREJ	**Not Detected N/A**	Not Detected	Any Result	Any Result

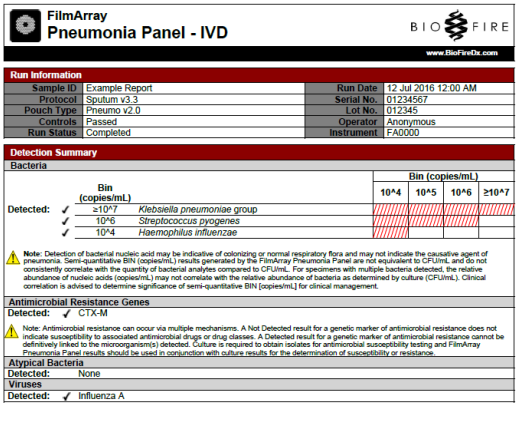
[a] Culturing and AST testing is required in order to assign a resistant and/or susceptible phenotype to isolates recovered from the BAL/Sputum specimens

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 Pneumonia Panel results should be used in conjunction with culture results for the determination of susceptibility or resistance.

NOTE: It is possible to obtain a Detected result for Staphylococcus aureus mecA/C and MREJ (MRSA) using the BIOFIRE Pneumonia Panel but to recover an isolate from culture that is characterized as methicillin sensitive S. aureus (MSSA) by phenotypic AST methods. This can occur when a sample contains a strain of S. aureus that carries the orfX gene (MREJ) with an empty SCCmec cassette (no mecA or mecC gene; phenotypically MSSA) in a co-culture with a second Staphylococcus species carrying the mecA or mecC gene. This may also be observed in instances of heterogeneous cultures of MRSA and MSSA.

BIOFIRE Pneumonia Panel Test Report

The two-page BIOFIRE Pneumonia Panel report is displayed upon the completion of a run and contains three sections – Run Information, Detection Summary, and Result Summary. It can be saved as a PDF file and/or printed if desired.



FilmArray Pneumonia Panel - IVD

BIOFIRE www.BioFireDx.com

Run Information

Sample ID	Example Report	Run Date	12 Jul 2016 12:00 AM
Protocol	Sputum v3.3	Serial No.	01234567
Pouch Type	Pneumo v2.0	Lot No.	012345
Controls	Passed	Operator	Anonymous
Run Status	Completed	Instrument	FA0000

Detection Summary

Bacteria

	Bin (copies/mL)		Bin (copies/mL)			
			10^4	10^5	10^6	≥10^7
Detected:	✓	≥10^7 *Klebsiella pneumoniae* group				
	✓	10^6 *Streptococcus pyogenes*				
	✓	10^4 *Haemophilus influenzae*				

⚠ Note: Detection of bacterial nucleic acid may be indicative of colonizing or normal respiratory flora and may not indicate the causative agent of pneumonia. Semi-quantitative BIN (copies/mL) results generated by the FilmArray Pneumonia Panel are not equivalent to CFU/mL and do not consistently correlate with the quantity of bacterial analytes compared to CFU/mL. For specimens with multiple bacteria detected, the relative abundance of nucleic acids (copies/mL) may not correlate with the relative abundance of bacteria as determined by culture (CFU/mL). Clinical correlation is advised to determine significance of semi-quantitative BIN [copies/mL] for clinical management.

Antimicrobial Resistance Genes

Detected: ✓ CTX-M

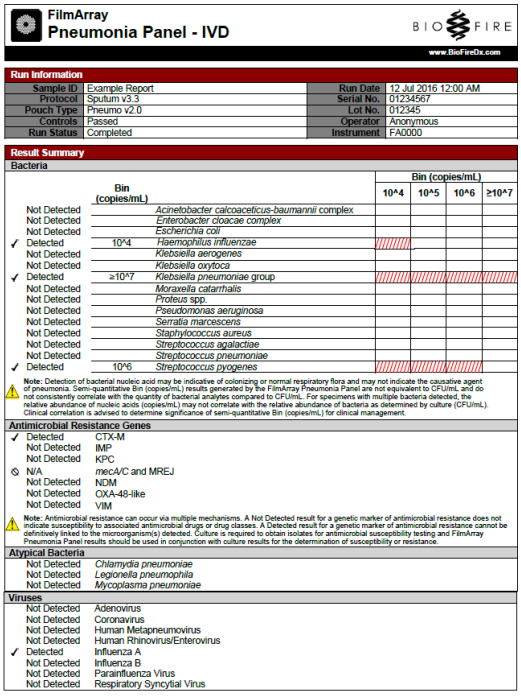
⚠ Note: Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for a genetic marker of antimicrobial resistance does not indicate susceptibility to associated antimicrobial drugs or drug classes. A Detected result for a genetic marker of antimicrobial resistance cannot be definitively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

Atypical Bacteria

Detected: None

Viruses

Detected: ✓ Influenza A



FilmArray Pneumonia Panel - IVD

BIOFIRE www.BioFireDx.com

Run Information

Sample ID	Example Report	Run Date	12 Jul 2016 12:00 AM
Protocol	Sputum v3.3	Serial No.	01234567
Pouch Type	Pneumo v2.0	Lot No.	012345
Controls	Passed	Operator	Anonymous
Run Status	Completed	Instrument	FA0000

Result Summary

Bacteria

	Bin (copies/mL)		Bin (copies/mL)			
			10^4	10^5	10^6	≥10^7
Not Detected		*Acinetobacter calcoaceticus-baumannii* complex				
Not Detected		*Enterobacter cloacae complex*				
Not Detected		*Escherichia coli*				
✓ Detected	10^4	*Haemophilus influenzae*				
Not Detected		*Klebsiella aerogenes*				
Not Detected		*Klebsiella oxytoca*				
✓ Detected	≥10^7	*Klebsiella pneumoniae* group				
Not Detected		*Moraxella catarrhalis*				
Not Detected		*Proteus* spp.				
Not Detected		*Pseudomonas aeruginosa*				
Not Detected		*Serratia marcescens*				
Not Detected		*Staphylococcus aureus*				
Not Detected		*Streptococcus agalactiae*				
Not Detected		*Streptococcus pneumoniae*				
✓ Detected	10^6	*Streptococcus pyogenes*				

⚠ Note: Detection of bacterial nucleic acid may be indicative of colonizing or normal respiratory flora and may not indicate the causative agent of pneumonia. Semi-quantitative Bin (copies/mL) results generated by the FilmArray Pneumonia Panel are not equivalent to CFU/mL and do not consistently correlate with the quantity of bacterial analytes compared to CFU/mL. For specimens with multiple bacteria detected, the relative abundance of nucleic acids (copies/mL) may not correlate with the relative abundance of bacteria as determined by culture (CFU/mL). Clinical correlation is advised to determine significance of semi-quantitative Bin (copies/mL) for clinical management.

Antimicrobial Resistance Genes

✓ Detected	CTX-M
Not Detected	IMP
Not Detected	KPC
⊘ N/A	*mecA/C* and MREJ
Not Detected	NDM
Not Detected	OXA-48-like
Not Detected	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 FilmArray Pneumonia Panel results should be used in conjunction with culture results for the determination of susceptibility or resistance.

Atypical Bacteria

Not Detected	*Chlamydia pneumoniae*
Not Detected	*Legionella pneumophila*
Not Detected	*Mycoplasma pneumoniae*

Viruses

Not Detected	Adenovirus
Not Detected	Coronavirus
Not Detected	Human Metapneumovirus
Not Detected	Human Rhinovirus/Enterovirus
✓ Detected	Influenza A
Not Detected	Influenza B
Not Detected	Parainfluenza Virus
Not Detected	Respiratory Syncytial Virus

Run Information

The Run Information section is displayed at the top of both pages of the test report. It provides information about the sample and the run including: Sample ID, Protocol (sample type), pouch information (Pouch Type, Lot Number, and Serial Number), Run Date, Run Status (Completed, Incomplete, Aborted, Instrument Error, Instrument Communication Error, or Software Error), the identity of the operator who performed the test, and the instrument used to perform the test. Control results are reported as Passed, Failed, or Invalid. Table 5 provides additional information for each of the possible control field results.

Table 5. Interpretation of Controls Field on the BIOFIRE Pneumonia Panel Test Report

Control Result	Explanation	Action
Passed	The run was successfully completed AND Both pouch controls were successful.	None Report the results provided on the test report.
Failed	The run was successfully completed BUT At least one of the pouch controls (RNA Process Control and/or QSM) failed.	Repeat the test using a new pouch. If the error persists, contact Customer 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 Information section of the report. Refer to the appropriate BIOFIRE Operator's Manual or contact Customer Technical Support for further instruction. Once the error is resolved, repeat the test or repeat the test using another instrument.

Detection Summary

The Detection Summary section is displayed on the first page of the report and lists the Detected results under each category (Bacteria, Antimicrobial Resistance Genes, Atypical Bacteria, and Viruses), including the semi-quantitative 'Bin (copies/mL)' results for Bacteria. If there are no Detected results in a specific category, the result shown is Detected: None.

Results Summary

The Results Summary is displayed on the second page of the report and provides a full list of test results for each organism and antimicrobial resistance gene including the 'Bin (copies/mL)' result for Bacteria. Possible results for each organism are Detected, Not Detected, Invalid, and N/A. Table 6 provides an explanation for each interpretation and any follow-up necessary to obtain a final result.

Table 6. Reporting of Results and Required Actions

Result	Explanation	Action
Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) for the organism were POSITIVE[a]	Report results.
Not Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) for the organism were NEGATIVE[b]	Report results.
Invalid	The pouch controls were not successful (Failed) OR The run was not successful (Run Status displayed as: Aborted, Incomplete, Instrument Error or Software Error)	See Table 5 for instruction.
N/A	The run was successfully completed	Report results.

Result	Explanation	Action
(Antimicrobial Resistance Genes only)	AND The pouch controls were successful (Passed) AND The assay(s) for the organism(s) associated with the antimicrobial resistance gene were NEGATIVE so the results of the antimicrobial resistance gene are not applicable to the test results.	

[a] For bacteria, the organism calculated value must be greater than or equal to 10^3.5 copies/mL for the assay to be POSITIVE.

[b] For bacteria, a NEGATIVE assay result may indicate no amplification or amplification with an organism calculated value less than 10^3.5 copies/mL

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 each page of 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.

Change Summary				
Field	Changed To	Changed From	Operator	Date
[1]Sample ID	Positive_example_XYZ	Positive _example	Jane Doe (JD)	16 Sept 2017

LIMITATIONS

1. For prescription use only.
2. The BIOFIRE Pneumonia Panel has not been validated for testing of specimens other than unprocessed sputum-like and BAL-like specimens.
3. Contact or treatment of specimens with decontaminating agents (bleach, MycoPrep (NaOH and NALC), 2% NaOH, and 5% Oxalic acid) can cause false negative results (see Interference section).
4. The performance of BIOFIRE Pneumonia Panel has not been established for specimens collected from individuals without signs and/or symptoms of lower respiratory infection.
5. The performance of the BIOFIRE Pneumonia Panel has not been established for monitoring treatment of infection.
6. The effect of antibiotic treatment on test performance including semi-quantitative bin results has not been specifically evaluated.
7. Viral and bacterial nucleic acids may persist *in vivo* independent of organism viability. Detection of organism target(s) does not imply that the corresponding organisms are infectious or are the causative agents for clinical symptoms.
8. The BIOFIRE Pneumonia Panel results for bacteria are provided as a qualitative Detected/Not Detected result with an associated semi-quantitative bin result of 10^4, 10^5, 10^6, or ≥10^7 copies of genomic nucleic acid per milliliter of specimen. An exact quantitative value is not provided. The semi-quantitative (copies/mL) bin result does not distinguish between nucleic acid from live or dead bacteria.
9. A negative BIOFIRE Pneumonia Panel result does not exclude the possibility of viral or bacterial infection. Negative test results may occur from the presence of 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 antiviral/antibacterial therapy or levels of organism in the specimen that are below the limit of detection for the test or below the reportable level for bacterial analytes. Negative results should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.
10. Concomitant culture of specimens is required with the BIOFIRE Pneumonia Panel. Culture is needed for recovery of isolates and antimicrobial susceptibility testing, as well as further speciation of genus, complex, or group level results (if desired).
11. Due to the genetic similarity between human rhinovirus and enterovirus, the BIOFIRE Pneumonia Panel cannot reliably differentiate them. A BIOFIRE Pneumonia Panel Human Rhinovirus/Enterovirus Detected result should be followed-up using an alternate method (e.g. cell culture or sequence analysis) if differentiation between the viruses is required.
12. The *in silico* analyses performed to predict amplification and detection of organisms and antimicrobial resistance genes were based on a comparison of target gene sequences available in GenBank to BIOFIRE Pneumonia Panel primer sequences. *In silico* analyses were performed between January 2016 and January 2018. Entries of new sequences added to the database after these dates have not been evaluated. Additional limitations on reactivity may be identified as new sequence data are deposited and/or as new sequence variants emerge.
13. Based on *in silico* analysis, the MREJ assay (which is only reported if *Staphylococcus aureus* is detected and the *mecA/C* assay is also positive) is predicted to have impaired reactivity or to be non-reactive with MREJ types ix, xv, and xviii, as well as types xix and xx (associated with methicillin-sensitive *S. aureus*; MSSA), and MREJ sequences annotated from non-*aureus Staphylococcus* species and non-Staphylococci such as *Bacillus cereus*, *Bacillus thuringiensis*, *Macrococcus caseolyticus*, *Clostridium acidurici*, and *Rummeliibacillus stabekisii*.

14. Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely during peak activity when prevalence of disease is high. False positive test results are more likely during periods when prevalence is moderate to low.
15. Performance characteristics for influenza A viruses were established during the 2016-2017 influenza season. When other novel influenza A viruses are emerging, performance characteristics may vary. If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
16. Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for several analytes in one or both matrices were primarily established using archived and/or contrived specimens as detailed in the Clinical Performance section.
17. There is an increased risk of false negative Adenovirus results for adenovirus species C when using a pouch that is within 6 months of the expiration date due to a 10-100 x loss in sensitivity (i.e. impairment leading to an increase in the LoD). The test performance is not impacted if kits are more than 6 months from expiration date. Performance for other adenovirus species is not impacted.
18. If using a pouch that is within 6 months of expiration when a patient is suspected of adenovirus C infection, confirm all negative Adenovirus results using another method prior to reporting the result, or alternatively, do not report a negative Adenovirus result.
19. False positives and false negatives can be the result of a variety of sources and causes. It is important that results be used in conjunction with other clinical, epidemiological, or laboratory information.

EXPECTED VALUES

In the prospective clinical evaluation of the BIOFIRE Pneumonia Panel, 846 BAL (including mini-BAL) and 836 sputum (including ETA) specimens, were collected and tested at eight study sites across the United States over approximately ten months (October 2016 to July 2017). Expected value (as determined by BIOFIRE Pneumonia Panel) summaries for BAL and sputum specimens are stratified by subject age and care setting in Table 7 through Table 12.

Table 7. Expected Value (As Determined by BIOFIRE Pneumonia Panel) Summary by Age Group for BAL Specimens Collected from Hospitalized Subjects During the BIOFIRE Pneumonia Panel Prospective Clinical Evaluation (October 2016 to July 2017)

BAL												
BIOFIRE Result	Overall (N=846)		Hospitalized (N=666)									
			≤5 (N=8)		6-17 (N=18)		18-34 (N=61)		35-65 (N=366)		>65 (N=212)	
	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV
Acinetobacter calcoaceticus-baumannii complex	7	0.8%	0	0%	0	0%	0	0%	4	1.1%	2	0.9%
Enterobacter cloacae complex	23	2.7%	0	0%	0	0%	0	0%	10	2.7%	12	5.7%
Escherichia coli	20	2.4%	0	0%	0	0%	3	4.9%	8	2.2%	7	3.3%
Haemophilus influenzae	82	9.7%	2	25.0%	6	33.3%	6	9.8%	38	10.4%	8	3.8%
Klebsiella aerogenes	13	1.5%	0	0%	0	0%	1	1.6%	4	1.1%	7	3.3%
Klebsiella oxytoca	11	1.3%	1	12.5%	0	0%	2	3.3%	5	1.4%	2	0.9%
Klebsiella pneumoniae group	27	3.2%	1	12.5%	0	0%	2	3.3%	10	2.7%	9	4.2%
Moraxella catarrhalis	29	3.4%	3	37.5%	1	5.6%	1	1.6%	10	2.7%	2	0.9%
Proteus spp.	9	1.1%	0	0%	0	0%	1	1.6%	2	0.5%	6	2.8%
Pseudomonas aeruginosa	74	8.7%	1	12.5%	2	11.1%	3	4.9%	30	8.2%	22	10.4%
Serratia marcescens	12	1.4%	0	0%	0	0%	1	1.6%	3	0.8%	4	1.9%
Staphylococcus aureus	116	13.7%	1	12.5%	1	5.6%	13	21.3%	61	16.7%	24	11.3%
Streptococcus agalactiae	25	3.0%	0	0%	0	0%	4	6.6%	15	4.1%	2	0.9%
Streptococcus pneumoniae	29	3.4%	0	0%	2	11.1%	1	1.6%	13	3.6%	5	2.4%
Streptococcus pyogenes	8	0.9%	0	0%	1	5.6%	2	3.3%	2	0.5%	0	0%
CTX-M	7	0.8%	0	0%	0	0%	0	0%	5	1.4%	2	0.9%
IMP	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
KPC	3	0.4%	0	0%	0	0%	0	0%	1	0.3%	1	0.5%
mecA/C and MREJ (MRSA)	46	5.4%	1	12.5%	1	5.6%	4	6.6%	25	6.8%	12	5.7%
NDM	1	0.1%	0	0%	0	0%	0	0%	1	0.3%	0	0%
OXA-48-like	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
VIM	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Chlamydia pneumoniae	1	0.1%	0	0%	0	0%	0	0%	1	0.3%	0	0%

BAL												
BIOFIRE Result	Overall (N=846)		Hospitalized (N=666)									
	#	EV	≤5 (N=8)		6-17 (N=18)		18-34 (N=61)		35-65 (N=366)		>65 (N=212)	
			#	EV	#	EV	#	EV	#	EV	#	EV
Legionella pneumophila	2	0.2%	0	0%	0	0%	1	1.6%	1	0.3%	0	0%
Mycoplasma pneumoniae	4	0.5%	0	0%	1	5.6%	0	0%	1	0.3%	1	0.5%
Adenovirus	8	0.9%	1	12.5%	0	0%	1	1.6%	4	1.1%	1	0.5%
Coronavirus	31	3.7%	0	0%	0	0%	0	0%	16	4.4%	6	2.8%
Human metapneumovirus	9	1.1%	0	0%	0	0%	1	1.6%	4	1.1%	1	0.5%
Human rhinovirus/enterovirus	64	7.6%	3	37.5%	4	22.2%	4	6.6%	25	6.8%	11	5.2%
Influenza A virus	15	1.8%	0	0%	0	0%	0	0%	6	1.6%	7	3.3%
Influenza B virus	7	0.8%	0	0%	1	5.6%	1	1.6%	4	1.1%	0	0%
Parainfluenza virus	18	2.1%	0	0%	0	0%	2	3.3%	10	2.7%	5	2.4%
Respiratory syncytial virus	4	0.5%	0	0%	0	0%	0	0%	1	0.3%	3	1.4%

Table 8. Expected Value (As Determined by BIOFIRE Pneumonia Panel) Summary by Age Group for Sputum Specimens Collected from Hospitalized Subjects During the BIOFIRE Pneumonia Panel Prospective Clinical Evaluation (October 2016 to July 2017)

Sputum												
BIOFIRE Result	Overall (N=836)		Hospitalized (N=682)									
	#	EV	≤5 (N=102)		6-17 (N=64)		18-34 (N=68)		35-65 (N=252)		>65 (N=196)	
			#	EV	#	EV	#	EV	#	EV	#	EV
Acinetobacter calcoaceticus-baumannii complex	28	3.3%	3	2.9%	3	4.7%	2	2.9%	4	1.6%	5	2.6%
Enterobacter cloacae complex	32	3.8%	7	6.9%	1	1.6%	1	1.5%	9	3.6%	7	3.6%
Escherichia coli	48	5.7%	3	2.9%	4	6.3%	7	10.3%	8	3.2%	16	8.2%
Haemophilus influenzae	107	12.8%	23	22.5%	7	10.9%	9	13.2%	25	9.9%	20	10.2%
Klebsiella aerogenes	12	1.4%	2	2.0%	1	1.6%	1	1.5%	3	1.2%	3	1.5%
Klebsiella oxytoca	19	2.3%	3	2.9%	1	1.6%	2	2.9%	5	2.0%	3	1.5%
Klebsiella pneumoniae group	65	7.8%	8	7.8%	3	4.7%	7	10.3%	16	6.3%	20	10.2%
Moraxella catarrhalis	75	9.0%	17	16.7%	5	7.8%	4	5.9%	9	3.6%	10	5.1%
Proteus spp.	23	2.8%	0	0%	1	1.6%	3	4.4%	2	0.8%	4	2.0%
Pseudomonas aeruginosa	160	19.1%	9	8.8%	14	21.9%	18	26.5%	32	12.7%	33	16.8%
Serratia marcescens	53	6.3%	4	3.9%	4	6.3%	5	7.4%	6	2.4%	8	4.1%
Staphylococcus aureus	204	24.4%	23	22.5%	14	21.9%	18	26.5%	54	21.4%	43	21.9%
Streptococcus agalactiae	43	5.1%	3	2.9%	5	7.8%	4	5.9%	12	4.8%	4	2.0%
Streptococcus pneumoniae	51	6.1%	12	11.8%	2	3.1%	3	4.4%	11	4.4%	7	3.6%
Streptococcus pyogenes	11	1.3%	0	0%	4	6.3%	0	0%	2	0.8%	2	1.0%

Sputum												
BIOFIRE Result	Overall (N=836)		Hospitalized (N=682)									
	#	EV	≤5 (N=102)		6-17 (N=64)		18-34 (N=68)		35-65 (N=252)		>65 (N=196)	
			#	EV	#	EV	#	EV	#	EV	#	EV
CTX-M	9	1.1%	1	1.0%	1	1.6%	1	1.5%	1	0.4%	2	1.0%
IMP	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
KPC	7	0.8%	0	0%	0	0%	1	1.5%	1	0.4%	4	2.0%
mecA/C and MREJ (MRSA)	107	12.8%	6	5.9%	7	10.9%	8	11.8%	32	12.7%	28	14.3%
NDM	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
OXA-48-like	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
VIM	2	0.2%	0	0%	0	0%	1	1.5%	1	0.4%	0	0%
Chlamydia pneumoniae	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Legionella pneumophila	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Mycoplasma pneumoniae	7	0.8%	1	1.0%	1	1.6%	0	0%	0	0%	0	0%
Adenovirus	16	1.9%	2	2.0%	1	1.6%	0	0%	6	2.4%	3	1.5%
Coronavirus	35	4.2%	3	2.9%	0	0%	2	2.9%	7	2.8%	11	5.6%
Human metapneumovirus	22	2.6%	4	3.9%	3	4.7%	0	0%	5	2.0%	5	2.6%
Human rhinovirus/enterovirus	112	13.4%	21	20.6%	7	10.9%	8	11.8%	19	7.5%	14	7.1%
Influenza A virus	16	1.9%	1	1.0%	3	4.7%	0	0%	1	0.4%	4	2.0%
Influenza B virus	14	1.7%	0	0%	1	1.6%	0	0%	5	2.0%	5	2.6%
Parainfluenza virus	30	3.6%	4	3.9%	4	6.3%	1	1.5%	9	3.6%	7	3.6%
Respiratory syncytial virus	48	5.7%	17	16.7%	2	3.1%	3	4.4%	6	2.4%	10	5.1%

Table 9. Expected Value (As Determined by BIOFIRE Pneumonia Panel) Summary by Age Group for BAL Specimens Collected from Outpatient Subjects During the BIOFIRE Pneumonia Panel Prospective Clinical Evaluation (October 2016 to July 2017)

BAL												
BIOFIRE Result	Overall (N=846)		Outpatient (N=159)									
	#	EV	≤5 (N=15)		6-17 (N=8)		18-34 (N=5)		35-65 (N=93)		>65 (N=38)	
			#	EV	#	EV	#	EV	#	EV	#	EV
Acinetobacter calcoaceticus-baumannii complex	7	0.8%	0	0%	0	0%	0	0%	0	0%	0	0%
Enterobacter cloacae complex	23	2.7%	0	0%	0	0%	0	0%	0	0%	0	0%
Escherichia coli	20	2.4%	0	0%	0	0%	0	0%	0	0%	0	0%
Haemophilus influenzae	82	9.7%	4	26.7%	1	12.5%	2	40.0%	8	8.6%	2	5.3%
Klebsiella aerogenes	13	1.5%	0	0%	0	0%	0	0%	0	0%	1	2.6%
Klebsiella oxytoca	11	1.3%	0	0%	0	0%	0	0%	1	1.1%	0	0%
Klebsiella pneumoniae group	27	3.2%	0	0%	0	0%	0	0%	2	2.2%	1	2.6%

BAL												
BIOFIRE Result	Overall (N=846)		Outpatient (N=159)									
	#	EV	≤5 (N=15)		6-17 (N=8)		18-34 (N=5)		35-65 (N=93)		>65 (N=38)	
			#	EV	#	EV	#	EV	#	EV	#	EV
Moraxella catarrhalis	29	3.4%	4	26.7%	1	12.5%	0	0%	4	4.3%	2	5.3%
Proteus spp.	9	1.1%	0	0%	0	0%	0	0%	0	0%	0	0%
Pseudomonas aeruginosa	74	8.7%	0	0%	0	0%	0	0%	8	8.6%	5	13.2%
Serratia marcescens	12	1.4%	0	0%	0	0%	0	0%	2	2.2%	0	0%
Staphylococcus aureus	116	13.7%	1	6.7%	0	0%	1	20.0%	6	6.5%	2	5.3%
Streptococcus agalactiae	25	3.0%	0	0%	0	0%	1	20.0%	2	2.2%	0	0%
Streptococcus pneumoniae	29	3.4%	2	13.3%	1	12.5%	1	20.0%	3	3.2%	1	2.6%
Streptococcus pyogenes	8	0.9%	1	6.7%	1	12.5%	0	0%	0	0%	0	0%
CTX-M	7	0.8%	0	0%	0	0%	0	0%	0	0%	0	0%
IMP	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
KPC	3	0.4%	0	0%	0	0%	0	0%	0	0%	0	0%
mecA/C and MREJ (MRSA)	46	5.4%	0	0%	0	0%	0	0%	1	1.1%	0	0%
NDM	1	0.1%	0	0%	0	0%	0	0%	0	0%	0	0%
OXA-48-like	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
VIM	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Chlamydia pneumoniae	1	0.1%	0	0%	0	0%	0	0%	0	0%	0	0%
Legionella pneumophila	2	0.2%	0	0%	0	0%	0	0%	0	0%	0	0%
Mycoplasma pneumoniae	4	0.5%	1	6.7%	0	0%	0	0%	0	0%	0	0%
Adenovirus	8	0.9%	0	0%	0	0%	0	0%	1	1.1%	0	0%
Coronavirus	31	3.7%	0	0%	0	0%	0	0%	7	7.5%	2	5.3%
Human metapneumovirus	9	1.1%	0	0%	0	0%	0	0%	2	2.2%	0	0%
Human rhinovirus/enterovirus	64	7.6%	3	20.0%	4	50.0%	0	0%	6	6.5%	4	10.5%
Influenza A virus	15	1.8%	0	0%	0	0%	0	0%	1	1.1%	1	2.6%
Influenza B virus	7	0.8%	0	0%	0	0%	0	0%	0	0%	0	0%
Parainfluenza virus	18	2.1%	0	0%	0	0%	0	0%	0	0%	1	2.6%
Respiratory syncytial virus	4	0.5%	0	0%	0	0%	0	0%	0	0%	0	0%

Table 10. Expected Value (As Determined by BIOFIRE Pneumonia Panel) Summary by Age Group for Sputum Specimens Collected from Outpatient Subjects During the BIOFIRE Pneumonia Panel Prospective Clinical Evaluation (October 2016 to July 2017)

Sputum												
BIOFIRE Result	Overall (N=836)		Outpatient (N=73)									
	#	EV	≤5 (N=13)		6-17 (N=21)		18-34 (N=7)		35-65 (N=18)		>65 (N=14)	
			#	EV	#	EV	#	EV	#	EV	#	EV
Acinetobacter calcoaceticus-baumannii complex	28	3.3%	1	7.7%	4	19.0%	1	14.3%	0	0%	0	0%
Enterobacter cloacae complex	32	3.8%	3	23.1%	1	4.8%	0	0%	0	0%	1	7.1%
Escherichia coli	48	5.7%	0	0%	1	4.8%	1	14.3%	0	0%	0	0%
Haemophilus influenzae	107	12.8%	3	23.1%	4	19.0%	0	0%	3	16.7%	3	21.4%
Klebsiella aerogenes	12	1.4%	0	0%	0	0%	0	0%	0	0%	0	0%
Klebsiella oxytoca	19	2.3%	3	23.1%	0	0%	0	0%	0	0%	0	0%
Klebsiella pneumoniae group	65	7.8%	0	0%	2	9.5%	0	0%	2	11.1%	2	14.3%
Moraxella catarrhalis	75	9.0%	6	46.2%	7	33.3%	2	28.6%	1	5.6%	0	0%
Proteus spp.	23	2.8%	1	7.7%	3	14.3%	0	0%	1	5.6%	0	0%
Pseudomonas aeruginosa	160	19.1%	3	23.1%	13	61.9%	4	57.1%	3	16.7%	5	35.7%
Serratia marcescens	53	6.3%	1	7.7%	7	33.3%	0	0%	2	11.1%	0	0%
Staphylococcus aureus	204	24.4%	7	53.8%	14	66.7%	2	28.6%	1	5.6%	2	14.3%
Streptococcus agalactiae	43	5.1%	1	7.7%	3	14.3%	1	14.3%	1	5.6%	1	7.1%
Streptococcus pneumoniae	51	6.1%	1	7.7%	2	9.5%	1	14.3%	0	0%	0	0%
Streptococcus pyogenes	11	1.3%	0	0%	1	4.8%	0	0%	0	0%	0	0%
CTX-M	9	1.1%	0	0%	1	4.8%	1	14.3%	0	0%	0	0%
IMP	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
KPC	7	0.8%	0	0%	0	0%	0	0%	1	5.6%	0	0%
mecA/C and MREJ (MRSA)	107	12.8%	2	15.4%	10	47.6%	1	14.3%	0	0%	1	7.1%
NDM	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
OXA-48-like	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
VIM	2	0.2%	0	0%	0	0%	0	0%	0	0%	0	0%
Chlamydia pneumoniae	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Legionella pneumophila	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Mycoplasma pneumoniae	7	0.8%	0	0%	0	0%	0	0%	0	0%	0	0%
Adenovirus	16	1.9%	0	0%	0	0%	0	0%	0	0%	1	7.1%
Coronavirus	35	4.2%	1	7.7%	1	4.8%	4	57.1%	1	5.6%	1	7.1%
Human metapneumovirus	22	2.6%	0	0%	0	0%	1	14.3%	0	0%	0	0%
Human rhinovirus/enterovirus	112	13.4%	5	38.5%	5	23.8%	2	28.6%	2	11.1%	4	28.6%
Influenza A virus	16	1.9%	0	0%	0	0%	0	0%	2	11.1%	1	7.1%

Sputum												
BIOFIRE Result	Overall (N=836)		Outpatient (N=73)									
	#	EV	≤5 (N=13)		6-17 (N=21)		18-34 (N=7)		35-65 (N=18)		>65 (N=14)	
			#	EV	#	EV	#	EV	#	EV	#	EV
Influenza B virus	14	1.7%	0	0%	0	0%	0	0%	0	0%	1	7.1%
Parainfluenza virus	30	3.6%	0	0%	0	0%	0	0%	0	0%	0	0%
Respiratory syncytial virus	48	5.7%	1	7.7%	0	0%	0	0%	1	5.6%	0	0%

Table 11. Expected Value (As Determined by BIOFIRE Pneumonia Panel) Summary by Age Group for BAL Specimens Collected from Emergency Department Subjects During the BIOFIRE Pneumonia Panel Prospective Clinical Evaluation (October 2016 to July 2017)

BAL												
BIOFIRE Result	Overall (N=846)		Emergency (N=21)									
	#	EV	≤5 (N=0)		6-17 (N=1)		18-34 (N=4)		35-65 (N=11)		>65 (N=5)	
			#	EV	#	EV	#	EV	#	EV	#	EV
Acinetobacter calcoaceticus-baumannii complex	7	0.8%	0	-	0	0%	0	0%	1	9.1%	0	0%
Enterobacter cloacae complex	23	2.7%	0	-	0	0%	0	0%	1	9.1%	0	0%
Escherichia coli	20	2.4%	0	-	0	0%	1	25.0%	1	9.1%	0	0%
Haemophilus influenzae	82	9.7%	0	-	0	0%	1	25.0%	2	18.2%	1	20.0%
Klebsiella aerogenes	13	1.5%	0	-	0	0%	0	0%	0	0%	0	0%
Klebsiella oxytoca	11	1.3%	0	-	0	0%	0	0%	0	0%	0	0%
Klebsiella pneumoniae group	27	3.2%	0	-	0	0%	0	0%	1	9.1%	0	0%
Moraxella catarrhalis	29	3.4%	0	-	0	0%	0	0%	0	0%	0	0%
Proteus spp.	9	1.1%	0	-	0	0%	0	0%	0	0%	0	0%
Pseudomonas aeruginosa	74	8.7%	0	-	0	0%	0	0%	2	18.2%	1	20.0%
Serratia marcescens	12	1.4%	0	-	0	0%	0	0%	0	0%	1	20.0%
Staphylococcus aureus	116	13.7%	0	-	1	100%	2	50.0%	3	27.3%	0	0%
Streptococcus agalactiae	25	3.0%	0	-	0	0%	0	0%	1	9.1%	0	0%
Streptococcus pneumoniae	29	3.4%	0	-	0	0%	0	0%	0	0%	0	0%
Streptococcus pyogenes	8	0.9%	0	-	0	0%	1	25.0%	0	0%	0	0%
CTX-M	7	0.8%	0	-	0	0%	0	0%	0	0%	0	0%
IMP	0	0%	0	-	0	0%	0	0%	0	0%	0	0%
KPC	3	0.4%	0	-	0	0%	0	0%	1	9.1%	0	0%
mecA/C and MREJ (MRSA)	46	5.4%	0	-	0	0%	1	25.0%	1	9.1%	0	0%
NDM	1	0.1%	0	-	0	0%	0	0%	0	0%	0	0%
OXA-48-like	0	0%	0	-	0	0%	0	0%	0	0%	0	0%
VIM	0	0%	0	-	0	0%	0	0%	0	0%	0	0%

BAL												
BIOFIRE Result	Overall (N=846)		Emergency (N=21)									
	#	EV	≤5 (N=0)		6-17 (N=1)		18-34 (N=4)		35-65 (N=11)		>65 (N=5)	
			#	EV	#	EV	#	EV	#	EV	#	EV
Chlamydia pneumoniae	1	0.1%	0	-	0	0%	0	0%	0	0%	0	0%
Legionella pneumophila	2	0.2%	0	-	0	0%	0	0%	0	0%	0	0%
Mycoplasma pneumoniae	4	0.5%	0	-	0	0%	0	0%	0	0%	0	0%
Adenovirus	8	0.9%	0	-	0	0%	0	0%	0	0%	0	0%
Coronavirus	31	3.7%	0	-	0	0%	0	0%	0	0%	0	0%
Human metapneumovirus	9	1.1%	0	-	0	0%	0	0%	1	9.1%	0	0%
Human rhinovirus/enterovirus	64	7.6%	0	-	0	0%	0	0%	0	0%	0	0%
Influenza A virus	15	1.8%	0	-	0	0%	0	0%	0	0%	0	0%
Influenza B virus	7	0.8%	0	-	0	0%	0	0%	1	9.1%	0	0%
Parainfluenza virus	18	2.1%	0	-	0	0%	0	0%	0	0%	0	0%
Respiratory syncytial virus	4	0.5%	0	-	0	0%	0	0%	0	0%	0	0%

Table 12. Expected Value (As Determined by BIOFIRE Pneumonia Panel) Summary by Age Group for Sputum Specimens Collected from Emergency Department Subjects During the BIOFIRE Pneumonia Panel Prospective Clinical Evaluation (October 2016 to July 2017)

Sputum												
BIOFIRE Result	Overall (N=836)		Emergency (N=81)									
	#	EV	≤5 (N=23)		6-17 (N=22)		18-34 (N=11)		35-65 (N=14)		>65 (N=11)	
			#	EV	#	EV	#	EV	#	EV	#	EV
Acinetobacter calcoaceticus-baumannii complex	28	3.3%	2	8.7%	1	4.5%	1	9.1%	0	0.0%	1	9.1%
Enterobacter cloacae complex	32	3.8%	1	4.3%	1	4.5%	0	0%	0	0%	0	0%
Escherichia coli	48	5.7%	0	0%	5	22.7%	1	9.1%	1	7.1%	1	9.1%
Haemophilus influenzae	107	12.8%	2	8.7%	4	18.2%	2	18.2%	1	7.1%	1	9.1%
Klebsiella aerogenes	12	1.4%	0	0%	0	0%	1	9.1%	1	7.1%	0	0%
Klebsiella oxytoca	19	2.3%	1	4.3%	0	0%	0	0%	1	7.1%	0	0%
Klebsiella pneumoniae group	65	7.8%	2	8.7%	2	9.1%	0	0%	0	0%	1	9.1%
Moraxella catarrhalis	75	9.0%	8	34.8%	5	22.7%	1	9.1%	0	0%	0	0%
Proteus spp.	23	2.8%	1	4.3%	4	18.2%	1	9.1%	1	7.1%	1	9.1%
Pseudomonas aeruginosa	160	19.1%	9	39.1%	8	36.4%	7	63.6%	1	7.1%	1	9.1%
Serratia marcescens	53	6.3%	7	30.4%	5	22.7%	3	27.3%	0	0%	1	9.1%
Staphylococcus aureus	204	24.4%	11	47.8%	10	45.5%	0	0%	2	14.3%	3	27.3%
Streptococcus agalactiae	43	5.1%	0	0%	3	13.6%	2	18.2%	1	7.1%	2	18.2%
Streptococcus pneumoniae	51	6.1%	1	4.3%	7	31.8%	1	9.1%	2	14.3%	1	9.1%

Sputum												
BIOFIRE Result	Overall (N=836)		Emergency (N=81)									
	#	EV	≤5 (N=23)		6-17 (N=22)		18-34 (N=11)		35-65 (N=14)		>65 (N=11)	
			#	EV	#	EV	#	EV	#	EV	#	EV
Streptococcus pyogenes	11	1.3%	0	0%	2	9.1%	0	0%	0	0%	0	0%
CTX-M	9	1.1%	0	0%	1	4.5%	0	0%	0	0%	0	0%
IMP	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
KPC	7	0.8%	0	0%	0	0%	0	0%	0	0%	0	0%
mecA/C and MREJ (MRSA)	107	12.8%	5	21.7%	4	18.2%	0	0%	0	0%	3	27.3%
NDM	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
OXA-48-like	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
VIM	2	0.2%	0	0%	0	0%	0	0%	0	0%	0	0%
Chlamydia pneumoniae	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Legionella pneumophila	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Mycoplasma pneumoniae	7	0.8%	0	0%	3	13.6%	1	9.1%	0	0%	1	9.1%
Adenovirus	16	1.9%	1	4.3%	1	4.5%	1	9.1%	0	0%	0	0%
Coronavirus	35	4.2%	1	4.3%	2	9.1%	1	9.1%	0	0%	0	0%
Human metapneumovirus	22	2.6%	1	4.3%	0	0%	1	9.1%	1	7.1%	1	9.1%
Human rhinovirus/enterovirus	112	13.4%	12	52.2%	7	31.8%	3	27.3%	1	7.1%	2	18.2%
Influenza A virus	16	1.9%	0	0%	1	4.5%	0	0%	2	14.3%	1	9.1%
Influenza B virus	14	1.7%	0	0%	1	4.5%	0	0%	0	0%	1	9.1%
Parainfluenza virus	30	3.6%	0	0%	3	13.6%	0	0%	2	14.3%	0	0%
Respiratory syncytial virus	48	5.7%	3	13.0%	3	13.6%	1	9.1%	1	7.1%	0	0%

In addition, observed multiple detections in each specimen type (as determined by BIOFIRE Pneumonia Panel) during the BIOFIRE Pneumonia Panel prospective clinical evaluation is presented in Table 13. The BIOFIRE Pneumonia Panel detected at least one analyte in a total of 413 BAL specimens (48.8% positivity rate; 413/846) and 602 sputum specimens (72.0% positivity rate; 602/836). Two or more analytes were detected by the BIOFIRE Pneumonia Panel in 37.8% of positive BAL specimens (156/413; 18.4% of all tested BAL specimens, 156/846) and 56.5% of positive sputum specimens (340/602; 40.7% of all tested sputum specimens, 340/836). Up to six analytes were detected in both specimen types.

Table 13. Expected Values (Multiple Detections as Determined by the BIOFIRE Pneumonia Panel) for the BIOFIRE Pneumonia Panel Clinical Evaluation (October 2016 – July 2017)

BIOFIRE Result	Expected Value (as Determined by Testing of 846 Prospective BAL Specimens)		Expected Value (as Determined by Testing of 836 Prospective Sputum Specimens)	
	Number Detected and Reported	% of Total (% of Positives)	Number Detected and Reported	% of Total (% of Positives)
Detected (at least one result)	413	48.8% (100%)	602	72.0% (100%)
One analyte result	257	30.4% (62.2%)	262	31.3% (43.5%)
Two analyte results	105	12.4% (25.4%)	178	21.3% (29.6%)
Three analyte results	28	3.3% (6.8%)	85	10.2% (14.1%)
Four analyte results	20	2.4% (4.8%)	42	5.0% (7.0%)
Five analyte results	2	0.2% (0.5%)	23	2.8% (3.8%)
Six or more analyte results	1	0.1% (0.2%)	12	1.4% (2.0%)

Detection profiles, including co-detections with multiple bacteria, multiple viruses, or bacteria and virus combinations, are shown in Figure 1. Viruses and bacteria were observed together in 6% of BAL and 21% of sputum specimens. The rate of multiple bacteria detections in single specimens was higher in sputum specimens (19%) compared to BAL (12%). Multiple viruses were observed in both specimen types at a low rate (1%).

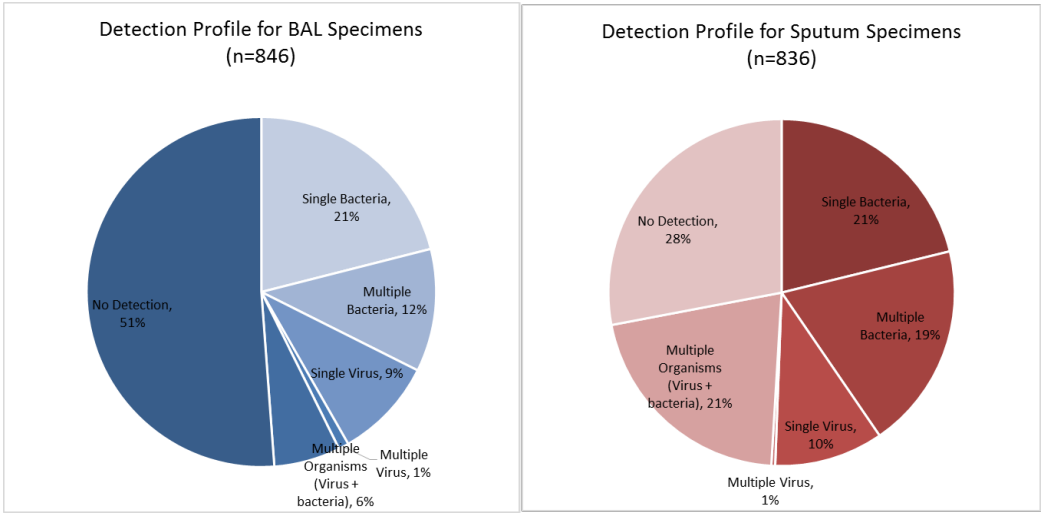


Figure 1. Detection profiles (as determined by the BIOFIRE Pneumonia Panel) for BAL and sputum specimens (AMR genes excluded)

The BIOFIRE Pneumonia Panel identified 119 different co-detection combinations in 156 BAL specimens, 100 of which were unique combinations (Table 14 and Table 15). False positive results (as compared to SOC [typical bacteria and AMR] and PCR/seq [atypical bacteria and virus]) were observed in 104 of 156 BAL specimens with co-detections. Similarly, 243 different co-detection combinations were identified in 340 sputum specimens, 194 of which were unique combinations. False positive results (as compared to SOC [typical bacteria and AMR] and PCR/seq [atypical bacteria and virus]) were observed in 239 of 340 sputum specimens with co-detections.

Table 14. Co-detections by BIOFIRE Pneumonia Panel with performance compared to SOC for BAL specimens

BAL					
Organism Co-Detections (includes viruses and bacteria)	**Number of Co-Detection Combinations**	**Number of Co-Detection Combinations Observed in only One Specimen**	**Total Specimens with Co-Detection**	**Total Specimens with False Positive(s)**	**False Positive Analyte(s)/Total Analytes**
Two analyte results	69	51	105	64	78/210
Three analyte results	27	26	28	20	34/84
Four analyte results	20	20	20	17	44/80
Five analyte results	2	2	2	2	6/10
Six analyte results	0	-	0	-	-
Seven analyte results	1	1	1	1	6/7
All co-detections	**119**	**100**	**156**	**104**	**168/391**

Table 15. Co-detections by BIOFIRE Pneumonia Panel with performance compared to SOC for sputum specimens

Sputum					
Organism Co-Detections (includes viruses and bacteria)	**Number of Co-Detection Combinations**	**Number of Co-Detection Combinations Observed in only One Specimen**	**Total Specimens with Co-Detection**	**Total Specimens with False Positive(s)**	**False Positive Analyte(s)/Total Analytes**
Two analyte results	91	52	178	104	128/356
Three analyte results	76	67	85	68	109/255
Four analyte results	41	40	42	34	79/168
Five analyte results	23	23	23	21	62/115
Six analyte results	9	9	9	9	31/54
Seven analyte results	3	3	3	3	14/21
All co-detections	**243**	**194**	**340**	**239**	**423/969**

SOC testing reported Normal Oral Flora (NOF) and no specific organism for 79/322 (24.5%) BAL and 141/510 (27.6%) sputum specimens for which the BIOFIRE Pneumonia Panel reported at least one non-atypical bacteria (Table 16 and Table 17).

Table 16. Non-Atypical Bacterial Detections (as compared to SOC) in BAL Specimens for the BIOFIRE Pneumonia Panel Clinical Evaluation (October 2016 – July 2017)

BAL				
BIOFIRE Pneumonia Panel Result (n=846)	**Positive SOC Culture**	**Negative SOC Culture**		**SOC Culture Not Performed**
		No Growth	**NOF Reported**	
Detected (n=322)	195/322 (60.6%)	43/322 (13.4%)	79/322 (24.5%)	5/322 (1.6%)
Not Detected (n=524)	11/524 (2.1%)	268/524 (51.1%)	242/524 (46.2%)	3/524 (0.6%)

Table 17. Non-Atypical Bacterial Detections (as compared to SOC) in Sputum Specimens for the BIOFIRE Pneumonia Panel Clinical Evaluation (October 2016 – July 2017)

Sputum				
BIOFIRE Pneumonia Panel Result (n=836)	**Positive SOC Culture**	**Negative SOC Culture**		**SOC Culture Not Performed**
		No Growth	**NOF Reported**	
Detected (n=510)	331/510 (64.9%)	26/510 (5.1%)	141/510 (27.6%)	12/510 (2.4%)
Not Detected (n=326)	11/326 (3.4%)	110/326 (33.7%)	201/326 (61.7%)	4/326 (1.2%)

Two or more non-atypical bacteria were detected by the BIOFIRE Pneumonia Panel in 42.8% (356/832) of positive specimens; 34.2% (110/322) of positive BAL specimens and 48.2% (246/510) of positive sputum specimens. The resulting co-detection combinations, as reported by the BIOFIRE Pneumonia Panel, are presented in Table 20 and Table 21. These tables also indicate the number of specimens with false positive results for each co-detection combination, as well as the specific analytes that were discrepant.

Table 18. Co-detections of Non-atypical Bacteria in BAL Detected by BIOFIRE Pneumonia Panel and Compared to qRefCx

Distinct Co-Detection Combinations							Total Specimens with Co-Detection Combination	Number of Specimens with False Positive Co-Detections	False Positive Analyte(s) [False Positive AMR Gene(s)[a]]
Analyte 1	**Analyte 2**	**Analyte 3**	**Analyte 4**	**Analyte 5**	**Analyte 6**	**AMR Gene(s)**			
H. influenzae	*K. oxytoca*	*M. catarrhalis*	*P. aeruginosa*	*S. aureus*	*S. agalactiae*	-	1	1	*H. influenzae, K. oxytoca, M. catarrhalis, S. aureus, S. agalactiae*
ACB complex	*K. aerogenes*	*Proteus* spp.	*P. aeruginosa*	*S. aureus*		*mecA/C* & MREJ (MRSA)	1	1	ACB complex
ACB complex	*E. cloacae* complex	*K. pneumoniae* group	*S. agalactiae*			-	1	1	ACB complex, *S. agalactiae*
ACB complex	*E. coli*	*H. influenzae*	*P. aeruginosa*			-	1	1	ACB complex, *H. influenzae*, *P. aeruginosa*

Distinct Co-Detection Combinations							Total Specimens with Co-Detection Combination	Number of Specimens with False Positive Co-Detections	False Positive Analyte(s) [False Positive AMR Gene(s)[a]]
Analyte 1	**Analyte 2**	**Analyte 3**	**Analyte 4**	**Analyte 5**	**Analyte 6**	**AMR Gene(s)**			
E. cloacae complex	*H. influenzae*	*K. oxytoca*	*K. pneumoniae* group			-	1	1	*E. cloacae* complex, *H. influenzae, K. oxytoca, K. pneumoniae* group
E. cloacae complex	*K. oxytoca*	*K. pneumoniae* group	*Proteus* spp.			-	1	1	*E. cloacae* complex, *K. oxytoca*
E. coli	*H. influenzae*	*K. aerogenes*	*S. aureus*			-	1	1	*E. coli, H. influenzae, K. aerogenes, S. aureus*
E. coli	*H. influenzae*	*S. aureus*	*S. agalactiae*			-	1	1	*E. coli, H. influenzae, S. agalactiae*
E. coli	*K. oxytoca*	*P. aeruginosa*	*S. marcescens*			-	1	1	*K. oxytoca, S. marcescens*
H. influenzae	*K. pneumoniae* group	*M. catarrhalis*	*S. marcescens*			-	1	1	*H. influenzae, M. catarrhalis*
H. influenzae	*Proteus* spp.	*S. aureus*	*S. pyogenes*			-	1	1	*H. influenzae, Proteus* spp., *S. aureus, S. pyogenes*
H. influenzae	*S. aureus*	*S. agalactiae*	*S. pneumoniae*			-	1	1	*H. influenzae, S. aureus, S. agalactiae, S. pneumoniae*
K. oxytoca	*P. aeruginosa*	*S. marcescens*	*S. aureus*			-	1	1	*S. marcescens*
K. pneumoniae group	*S. aureus*	*S. agalactiae*	*S. pneumoniae*			-	1	1	*S. aureus, S. agalactiae, S. pneumoniae*
ACB complex	*H. influenzae*	*S. pneumoniae*				-	1	1	ACB complex, *H. influenzae, S. pneumoniae*
E. cloacae complex	*H. influenzae*	*K. oxytoca*				-	1	1	*H. influenzae*
E. cloacae complex	*H. influenzae*	*S. pneumoniae*				-	1	1	*H. influenzae, S. pneumoniae*
E. coli	*H. influenzae*	*S. pneumoniae*				-	1	1	*E. coli, S. pneumoniae*
E. coli	*K. pneumoniae* group	*S. pneumoniae*				-	1	1	*K. pneumoniae* group, *S. pneumoniae*
H. influenzae	*K. pneumoniae* group	*M. catarrhalis*				-	1	1	*H. influenzae, K. pneumoniae* group, *M. catarrhalis*

Distinct Co-Detection Combinations							Total Specimens with Co-Detection Combination	Number of Specimens with False Positive Co-Detections	False Positive Analyte(s) [False Positive AMR Gene(s)[a]]
Analyte 1	**Analyte 2**	**Analyte 3**	**Analyte 4**	**Analyte 5**	**Analyte 6**	**AMR Gene(s)**			
H. influenzae	*K. pneumoniae* group	*P. aeruginosa*				-	1	1	*H. influenzae*
H. influenzae	*M. catarrhalis*	*S. aureus*				-	2	2	*H. influenzae* (1), *M. catarrhalis* (2)
H. influenzae	*M. catarrhalis*	*S. pneumoniae*				-	2	2	*H. influenzae* (2), *M. catarrhalis* (2), *S. pneumoniae* (2)
H. influenzae	*S. aureus*	*S. agalactiae*				-	1	1	*H. influenzae, S. aureus, S. agalactiae*
H. influenzae	*S. aureus*	*S. pyogenes*				*mecA/C* & MREJ (MRSA)	1	1	*H. influenzae, S. aureus, S. pyogenes*
K. aerogenes	*P. aeruginosa*	*S. aureus*				-	1	1	*K. aerogenes, S. aureus*
K. pneumoniae group	*P. aeruginosa*	*S. marcescens*				-	1	1	*S. marcescens*
Proteus spp.	*P. aeruginosa*	*S. agalactiae*				KPC	1	1	*Proteus* spp., *P. aeruginosa, S. agalactiae*
P. aeruginosa	*S. marcescens*	*S. aureus*				-	1	1	*P. aeruginosa*
P. aeruginosa	*S. marcescens*	*S. pneumoniae*				-	1	0	-
S. marcescens	*S. aureus*	*S. agalactiae*				-	1	1	*S. marcescens, S. agalactiae*
S. aureus	*S. agalactiae*	*S. pneumoniae*				*mecA/C* & MREJ (MRSA)	1	1	*S. aureus, S. agalactiae, S. pneumoniae*
S. aureus	*S. agalactiae*	*S. pneumoniae*				-	1	1	*S. agalactiae, S. pneumoniae*
S. aureus	*S. pneumoniae*	*S. pyogenes*				-	1	1	*S. pneumoniae*
ACB complex	*K. pneumoniae* group					KPC	1	1	ACB complex, *K. pneumoniae* group, [KPC]
ACB complex	*P. aeruginosa*					-	1	1	ACB complex, *P. aeruginosa*
E. cloacae complex	*K. aerogenes*					-	1	1	*K. aerogenes*
E. cloacae complex	*K. oxytoca*					CTX-M[b]	1	1	*K. oxytoca*
E. cloacae complex	*K. pneumoniae* group					-	2	2	*E. cloacae* complex (2), *K. pneumoniae* group (2)

Distinct Co-Detection Combinations							Total Specimens with Co-Detection Combination	Number of Specimens with False Positive Co-Detections	False Positive Analyte(s) [False Positive AMR Gene(s)[a]]
Analyte 1	Analyte 2	Analyte 3	Analyte 4	Analyte 5	Analyte 6	AMR Gene(s)			
E. cloacae complex	*P. aeruginosa*					KPC	1	0	-
E. cloacae complex	*P. aeruginosa*					-	1	1	*E. cloacae* complex, *P. aeruginosa*
E. cloacae complex	*S. aureus*					NDM	1	1	*E. cloacae* complex, [NDM]
E. cloacae complex	*S. aureus*					-	1	1	*E. cloacae* complex, *S. aureus*
E. cloacae complex	*S. pneumoniae*					-	1	1	*E. cloacae complex, S. pneumoniae*
E. coli	*K. aerogenes*					-	1	1	*E. coli*
E. coli	*K. pneumoniae* group					-	1	0	-
E. coli	*S. aureus*					CTX-M, *mecA/C* & MREJ (MRSA)	1	1	*E. coli, S. aureus*
E. coli	*S. aureus*					*mecA/C* & MREJ (MRSA)	1	1	*E. coli, S. aureus*
E. coli	*S. aureus*					-	3	2	*E. coli* (1), *S. aureus* (1)
H. influenzae	*K. aerogenes*					-	1	1	*H. influenzae, K. aerogenes*
H. influenzae	*K. pneumoniae* group					-	1	1	*H. influenzae*
H. influenzae	*M. catarrhalis*					-	5	5	*H. influenzae* (4), *M. catarrhalis* (5)
H. influenzae	*P. aeruginosa*					-	1	1	*H. influenzae*
H. influenzae	*S. marcescens*					-	1	0	-
H. influenzae	*S. aureus*					*mecA/C* & MREJ (MRSA)	3	3	*H. influenzae* (3), *S. aureus* (1)
H. influenzae	*S. aureus*					-	6	5	*H. influenzae* (4), *S. aureus* (2)
H. influenzae	*S. agalactiae*					-	1	1	*H. influenzae, S. agalactiae*
H. influenzae	*S. pneumoniae*					-	7	7	*H. influenzae* (7), *S. pneumoniae* (3)
H. influenzae	*S. pyogenes*					-	1	1	*H. influenzae, S. pyogenes*
K. aerogenes	*S. aureus*					*mecA/C* & MREJ (MRSA)	2	2	K. aerogenes (2), S. aureus (1)

Distinct Co-Detection Combinations							Total Specimens with Co-Detection Combination	Number of Specimens with False Positive Co-Detections	False Positive Analyte(s) [False Positive AMR Gene(s)[a]]
Analyte 1	**Analyte 2**	**Analyte 3**	**Analyte 4**	**Analyte 5**	**Analyte 6**	**AMR Gene(s)**			
K. aerogenes	*S. aureus*					-	1	1	*S. aureus*
K. oxytoca	*P. aeruginosa*					-	1	1	*K. oxytoca*
K. oxytoca	*S. agalactiae*					-	2	2	*K. oxytoca* (2), *S. agalactiae* (2)
K. pneumoniae group	*S. aureus*					*mecA/C* & MREJ (MRSA)	1	1	*K. pneumoniae* group
K. pneumoniae group	*S. aureus*					-	1	1	*K. pneumoniae* group, *S. aureus*
M. catarrhalis	*Proteus* spp.					-	1	1	*M. catarrhalis*
M. catarrhalis	*S. pneumoniae*					-	1	1	*M. catarrhalis, S. pneumoniae*
M. catarrhalis	*S. pyogenes*					-	1	1	*M. catarrhalis, S. pyogenes*
Proteus spp.	*S. aureus*					*mecA/C* & MREJ (MRSA)	1	1	*S. aureus*, [*mecA/C* & MREJ]
P. aeruginosa	*S. aureus*					*mecA/C* & MREJ (MRSA)	5	4	*P. aeruginosa* (3), *S. aureus* (2), [*mecA/C* & MREJ (MRSA)]
P. aeruginosa	*S. aureus*					-	2	2	*S. aureus* (2)
P. aeruginosa	*S. agalactiae*					-	1	1	*P. aeruginosa, S. agalactiae*
P. aeruginosa	*S. pneumoniae*					-	1	1	*P. aeruginosa, S. pneumoniae*
S. marcescens	*S. aureus*					*mecA/C* & MREJ (MRSA)	1	1	*S. marcescens*
S. marcescens	*S. aureus*					-	1	1	*S. marcescens, S. aureus*
S. aureus	*S. agalactiae*					*mecA/C* & MREJ (MRSA)	1	1	*S. agalactiae*
S. aureus	*S. agalactiae*					-	4	4	*S. aureus* (4), *S. agalactiae* (3)
S. aureus	*S. pneumoniae*					*mecA/C* & MREJ (MRSA)	1	1	*S. aureus, S. pneumoniae*
Total Co-Detections							**110**	**103**	**187[c]/273**
Total Double Detections							**74**	**68**	**103/148**
Total Triple Detections							**22**	**21**	**44/66**
Total Quadruple Detections							**12**	**12**	**34/48**
Total Quintuple Detections							**1**	**1**	**1/5**
Total Sextuple Detections							**1**	**1**	**5/6**

[a] AMR genes compared to qMol.

[b] Performance not determined.

[c] Of the 187 discrepant analytes (out of 273 total analytes), all 187 (100%) were observed as being present in the specimen during discrepancy investigation; 55/187 (29.4%) were enumerated below 10^3.5 CFU/mL by qRefCx, 112/187 (59.9%) were detected by qMol, 17/187 (9.1%) were detected using an additional molecular method, and the remaining 3/187 (1.6%) were identified in SOC culture.

Table 19. Co-detections of Non-atypical Bacteria in Sputum Detected by BIOFIRE Pneumonia Panel and Compared to qRefCx

Distinct Co-Detection Combinations								Total Specimens with Co-Detection Combination	Number of Specimens with False Positive Co-Detections	False Positive Analyte(s) [False Positive AMR Gene(s)[a]]
Analyte 1	**Analyte 2**	**Analyte 3**	**Analyte 4**	**Analyte 5**	**Analyte 6**	**Analyte 7**	**AMR Gene(s)**			
ACB complex	*Proteus* spp.	*P. aeruginosa*	*S. marcescens*	*S. aureus*	*S. agalactiae*	*S. pneumoniae*	*mecA/C* & MREJ (MRSA)	1	1	ACB complex, *S. agalactiae*
E. coli	*K. pneumoniae* group	*M. catarrhalis*	*Proteus* spp.	*P. aeruginosa*	*S. aureus*	*S. agalactiae*	*mecA/C* & MREJ (MRSA)	1	1	*E. coli, K. pneumoniae* group, *M. catarrhalis, Proteus* spp., *P. aeruginosa, S. aureus, S. agalactiae*
H. influenzae	*K. pneumoniae* group	*P. aeruginosa*	*S. marcescens*	*S. aureus*	*S. agalactiae*		-	1	1	*H. influenzae, K. pneumoniae* group
M. catarrhalis	*Proteus* spp.	*P. aeruginosa*	*S. marcescens*	*S. aureus*	*S. agalactiae*		*mecA/C* & MREJ (MRSA)	1	1	*M. catarrhalis, P. aeruginosa, S. aureus, S. agalactiae*
M. catarrhalis	*Proteus* spp.	*P. aeruginosa*	*S. aureus*	*S. agalactiae*	*S. pneumoniae*		CTX-M, *mecA/C* & MREJ (MRSA)	1	1	*M. catarrhalis, Proteus* spp., *S. aureus, S. agalactiae, S. pneumoniae,* [*mecA/C* & MREJ (MRSA)]
ACB complex	*E. cloacae* complex	*K. aerogenes*	*K. pneumoniae* group	*S. aureus*			-	1	1	*ACB complex, K. aerogenes, K. pneumoniae group, S. aureus*
ACB complex	*K. aerogenes*	*K. pneumoniae* group	*P. aeruginosa*	*S. aureus*			KPC, *mecA/C* & MREJ (MRSA), VIM	1	1	*K. aerogenes, S. aureus, [mecA/C* & MREJ (MRSA)*]*
ACB complex	*M. catarrhalis*	*P. aeruginosa*	*S. aureus*	*S. agalactiae*			*mecA/C* & MREJ (MRSA)	1	1	*M. catarrhalis, P. aeruginosa, S. agalactiae*
E. cloacae complex	*E. coli*	*K. aerogenes*	*K. pneumoniae* group	*M. catarrhalis*			-	1	1	*E. cloacae complex, K. aerogenes, K. pneumoniae group, M. catarrhalis*
E. cloacae complex	*K. oxytoca*	*K. pneumoniae* group	*S. marcescens*	*S. aureus*			-	1	1	*E. cloacae* complex, *K. oxytoca, K. pneumoniae* group, *S. marcescens*
E. coli	*H. influenzae*	*K. oxytoca*	*P. aeruginosa*	*S. agalactiae*			-	1	1	*E. coli, H. influenzae, K. oxytoca, P. aeruginosa, S. agalactiae*
E. coli	*K. oxytoca*	*S. marcescens*	*S. aureus*	*S. agalactiae*			*mecA/C* & MREJ (MRSA)	1	1	*K. oxytoca, S. marcescens, S. agalactiae*

Distinct Co-Detection Combinations								Total Specimens with Co-Detection Combination	Number of Specimens with False Positive Co-Detections	False Positive Analyte(s) [False Positive AMR Gene(s)[a]]
Analyte 1	**Analyte 2**	**Analyte 3**	**Analyte 4**	**Analyte 5**	**Analyte 6**	**Analyte 7**	**AMR Gene(s)**			
E. coli	*M. catarrhalis*	*P. aeruginosa*	*S. agalactiae*	*S. pneumoniae*			-	1	1	*M. catarrhalis*
H. influenzae	*M. catarrhalis*	*P. aeruginosa*	*S. marcescens*	*S. agalactiae*			-	1	1	*H. influenzae, M. catarrhalis, P. aeruginosa, S. marcescens, S. agalactiae*
M. catarrhalis	*Proteus* spp.	*P. aeruginosa*	*S. marcescens*	*S. aureus*			*mecA/C* & MREJ (MRSA)	1	1	*M. catarrhalis, P. aeruginosa, S. marcescens, S. aureus*
M. catarrhalis	*P. aeruginosa*	*S. marcescens*	*S. aureus*	*S. pneumoniae*			*mecA/C* & MREJ (MRSA)	2	2	*M. catarrhalis* (2), *P. aeruginosa* (2), *S. marcescens* (1)
ACB complex	*E. cloacae* complex	*K. oxytoca*	*S. aureus*				-	1	1	ACB complex, *E. cloacae* complex
ACB complex	*E. coli*	*K. pneumoniae* group	*P. aeruginosa*				-	1	1	ACB complex, *E. coli, K. pneumoniae* group
ACB complex	*E. coli*	*S. aureus*	*S. agalactiae*				-	1	1	ACB complex, *E. coli, S. aureus, S. agalactiae*
ACB complex	*K. pneumoniae* group	*M. catarrhalis*	*S. aureus*				*mecA/C* & MREJ (MRSA)	1	1	*M. catarrhalis*
ACB complex	*K. pneumoniae* group	*Proteus* spp.	*P. aeruginosa*				CTX-M, KPC	1	1	ACB complex, *Proteus* spp.
ACB complex	*M. catarrhalis*	*P. aeruginosa*	*S. aureus*				-	1	1	ACB complex, *M. catarrhalis, P. aeruginosa, S. aureus*
ACB complex	*Proteus* spp.	*P. aeruginosa*	*S. marcescens*				-	1	1	ACB complex, *S. marcescens*
E. cloacae complex	*K. oxytoca*	*K. pneumoniae* group	*S. marcescens*				-	1	1	*E. cloacae* complex, *K. oxytoca, S. marcescens*
E. cloacae complex	*K. pneumoniae* group	*P. aeruginosa*	*S. aureus*				-	1	1	*E. cloacae* complex
E. coli	*H. influenzae*	*S. aureus*	*S. pneumoniae*				-	1	1	*H. influenzae, S. aureus, S. pneumoniae*
E. coli	*K. pneumoniae* group	*M. catarrhalis*	*S. aureus*				*mecA/C* & MREJ (MRSA)	1	1	*E. coli, K. pneumoniae* group, *M. catarrhalis, S. aureus*
E. coli	*K. pneumoniae* group	*P. aeruginosa*	*S. aureus*				-	1	1	*K. pneumoniae* group

Distinct Co-Detection Combinations								Total Specimens with Co-Detection Combination	Number of Specimens with False Positive Co-Detections	False Positive Analyte(s) [False Positive AMR Gene(s)[a]]
Analyte 1	**Analyte 2**	**Analyte 3**	**Analyte 4**	**Analyte 5**	**Analyte 6**	**Analyte 7**	**AMR Gene(s)**			
E. coli	*Proteus* spp.	*P. aeruginosa*	*S. aureus*				*mecA/C* & MREJ (MRSA)	1	0	-
E. coli	*P. aeruginosa*	*S. marcescens*	*S. pneumoniae*				-	1	1	*E. coli, P. aeruginosa, S. marcescens, S. pneumoniae*
H. influenzae	*K. aerogenes*	*P. aeruginosa*	*S. pyogenes*				-	1	1	*K. aerogenes*
H. influenzae	*M. catarrhalis*	*P. aeruginosa*	*S. aureus*				*mecA/C* & MREJ (MRSA)	1	1	*H. influenzae, M. catarrhalis, P. aeruginosa,* [*mecA/C* & MREJ]
H. influenzae	*M. catarrhalis*	*P. aeruginosa*	*S. marcescens*				-	1	1	*M. catarrhalis*
H. influenzae	*P. aeruginosa*	*S. marcescens*	*S. aureus*				-	1	1	*H. influenzae, S. marcescens, S. aureus*
H. influenzae	*P. aeruginosa*	*S. marcescens*	*S. pneumoniae*				-	1	0	-
H. influenzae	*S. aureus*	*S. agalactiae*	*S. pneumoniae*				-	1	1	*H. influenzae, S. agalactiae, S. pneumoniae*
K. pneumoniae group	*P. aeruginosa*	*S. marcescens*	*S. aureus*				-	1	1	*K. pneumoniae* group, *S. aureus*
M. catarrhalis	*P. aeruginosa*	*S. marcescens*	*S. aureus*				*mecA/C* & MREJ (MRSA)	1	1	*M. catarrhalis*
M. catarrhalis	*P. aeruginosa*	*S. marcescens*	*S. aureus*				-	1	1	*M. catarrhalis, P. aeruginosa, S. marcescens, S. aureus*
ACB complex	*E. cloacae* complex	*S. aureus*					*mecA/C* & MREJ (MRSA)	1	1	*E. cloacae* complex
ACB complex	*H. influenzae*	*S. aureus*					-	1	1	ACB complex, *H. influenzae, S. aureus*
ACB complex	*K. pneumoniae* group	*P. aeruginosa*					-	1	1	ACB complex, *K. pneumoniae* group
ACB complex	*Proteus* spp.	*S. aureus*					*mecA/C* & MREJ (MRSA)	1	0	-
ACB complex	*P. aeruginosa*	*S. marcescens*					-	1	1	ACB complex, *P. aeruginosa, S. marcescens*
ACB complex	*P. aeruginosa*	*S. aureus*					*mecA/C* & MREJ (MRSA)	2	2	ACB complex (2), *P. aeruginosa* (1), *S. aureus* (1)
ACB complex	*P. aeruginosa*	*S. aureus*					-	1	1	ACB complex, *S. aureus*
ACB complex	*P. aeruginosa*	*S. agalactiae*					-	1	0	-

Distinct Co-Detection Combinations								Total Specimens with Co-Detection Combination	Number of Specimens with False Positive Co-Detections	False Positive Analyte(s) [False Positive AMR Gene(s)[a]]
Analyte 1	**Analyte 2**	**Analyte 3**	**Analyte 4**	**Analyte 5**	**Analyte 6**	**Analyte 7**	**AMR Gene(s)**			
E. cloacae complex	*E. coli*	*K. oxytoca*					-	1	1	*E. cloacae* complex, *K. oxytoca*
E. cloacae complex	*H. influenzae*	*P. aeruginosa*					-	1	1	*H. influenzae, P. aeruginosa*
E. cloacae complex	*H. influenzae*	*M. catarrhalis*					-	1	1	*H. influenzae, M. catarrhalis*
E. cloacae complex	*K. aerogenes*	*K. pneumoniae* group					-	1	1	*E. cloacae* complex, *K. pneumoniae* group
E. cloacae complex	*K. oxytoca*	*M. catarrhalis*					-	1	1	*E. cloacae* complex, *M. catarrhalis*
E. cloacae complex	*K. pneumoniae* group	*S. aureus*					-	1	1	*E. cloacae* complex, *K. pneumoniae* group, *S. aureus*
E. cloacae complex	*M. catarrhalis*	*S. aureus*					-	1	1	*M. catarrhalis, S. aureus*
E. cloacae complex	*P. aeruginosa*	*S. aureus*					*mecA/C* & MREJ (MRSA)	1	1	*E. cloacae* complex
E. cloacae complex	*P. aeruginosa*	*S. aureus*					-	1	1	*E. cloacae* complex
E. coli	*H. influenzae*	*P. aeruginosa*					-	1	1	*E. coli, H. influenzae, P. aeruginosa*
E. coli	*H. influenzae*	*S. aureus*					CTX-M	1	1	*H. influenzae, S. aureus*
E. coli	*H. influenzae*	*S. agalactiae*					-	1	1	*E. coli, H. influenzae, S. agalactiae*
E. coli	*K. pneumoniae* group	*P. aeruginosa*					CTX-M	1	1	*K. pneumoniae* group
E. coli	*K. pneumoniae* group	*P. aeruginosa*					-	1	1	*E. coli, K. pneumoniae* group, *P. aeruginosa*
E. coli	*K. pneumoniae* group	*S. aureus*					*mecA/C* & MREJ (MRSA)	1	1	*E. coli, K. pneumoniae* group
E. coli	*K. pneumoniae* group	*S. aureus*					-	1	1	*S. aureus*
E. coli	*M. catarrhalis*	*P. aeruginosa*					-	1	1	*E. coli, M. catarrhalis*
E. coli	*Proteus* spp.	*P. aeruginosa*					-	1	1	*E. coli, P. aeruginosa*
E. coli	*P. aeruginosa*	*S. aureus*					*mecA/C* & MREJ (MRSA)	1	1	*P. aeruginosa*

Distinct Co-Detection Combinations								Total Specimens with Co-Detection Combination	Number of Specimens with False Positive Co-Detections	False Positive Analyte(s) [False Positive AMR Gene(s)[a]]
Analyte 1	Analyte 2	Analyte 3	Analyte 4	Analyte 5	Analyte 6	Analyte 7	AMR Gene(s)			
E. coli	*P. aeruginosa*	*S. aureus*					-	1	0	-
H. influenzae	*K. pneumoniae* group	*S. aureus*						2	2	*H. influenzae* (1), *K. pneumoniae* group[b] (1), *S. aureus* (2)
H. influenzae	*M. catarrhalis*	*P. aeruginosa*					-	2	2	*H. influenzae* (2), *M. catarrhalis* (2), *P. aeruginosa*(1)
H. influenzae	*M. catarrhalis*	*S. aureus*					*mecA/C* & MREJ (MRSA)	1	1	*H. influenzae, M. catarrhalis, S. aureus*
H. influenzae	*M. catarrhalis*	*S. aureus*					-	1	1	*H. influenzae, S. aureus*
H. influenzae	*M. catarrhalis*	*S. agalactiae*					-	1	1	*H. influenzae, M. catarrhalis, S. agalactiae*
H. influenzae	*M. catarrhalis*	*S. pneumoniae*					-	3	3	*H. influenzae* (2), *M. catarrhalis* (3), *S. pneumoniae* (2)
H. influenzae	*P. aeruginosa*	*S. marcescens*					-	1	1	*H. influenzae, S. marcescens*
H. influenzae	*P. aeruginosa*	*S. aureus*					-	2	2	*H. influenzae* (2), *P. aeruginosa* (2), *S. aureus* (1)
H. influenzae	*P. aeruginosa*	*S. pneumoniae*					-	1	1	*H. influenzae, P. aeruginosa, S. pneumoniae*
H. influenzae	*S. marcescens*	*S. pneumoniae*					-	1	1	*H. influenzae, S. pneumoniae*
H. influenzae	*S. aureus*	*S. agalactiae*					-	1	1	*H. influenzae, S. aureus, S. agalactiae*
H. influenzae	*S. aureus*	*S. pneumoniae*					*mecA/C* & MREJ	1	1	*H. influenzae*
H. influenzae	*S. aureus*	*S. pyogenes*					-	1	1	*H. influenzae*
H. influenzae	*S. agalactiae*	*S. pneumoniae*					-	1	1	*S. agalactiae*
K. aerogenes	*Proteus* spp.	*P. aeruginosa*					-	1	1	*P. aeruginosa*
K. oxytoca	*P. aeruginosa*	*S. aureus*					*mecA/C* & MREJ (MRSA)	1	0	-
K. oxytoca	*S. marcescens*	*S. aureus*					-	1	0	-
K. oxytoca	*S. aureus*	*S. agalactiae*					*mecA/C* & MREJ (MRSA)	1	1	*K. oxytoca, S. agalactiae*
K. pneumoniae group	*P. aeruginosa*	*S. aureus*					*mecA/C* & MREJ (MRSA)	2	2	*K. pneumoniae* group (2), *P. aeruginosa* (1)

Distinct Co-Detection Combinations								Total Specimens with Co-Detection Combination	Number of Specimens with False Positive Co-Detections	False Positive Analyte(s) [False Positive AMR Gene(s)[a]]
Analyte 1	**Analyte 2**	**Analyte 3**	**Analyte 4**	**Analyte 5**	**Analyte 6**	**Analyte 7**	**AMR Gene(s)**			
K. pneumoniae group	*P. aeruginosa*	*S. aureus*					-	1	0	-
K. pneumoniae group	*S. marcescens*	*S. pneumoniae*					-	1	1	*K. pneumoniae* group, *S. marcescens, S. pneumoniae*
K. pneumoniae group	*S. aureus*	*S. agalactiae*					-	1	1	*S. agalactiae*
M. catarrhalis	*Proteus* spp.	*S. agalactiae*					-	1	1	*M. catarrhalis, Proteus* spp.
M. catarrhalis	*P. aeruginosa*	*S. marcescens*					-	1	1	*M. catarrhalis, S. marcescens*
M. catarrhalis	*P. aeruginosa*	*S. aureus*					CTX-M, *mecA/C* & MREJ (MRSA)	1	1	*M. catarrhalis, S. aureus*
M. catarrhalis	*S. aureus*	*S. pneumoniae*					*mecA/C* & MREJ (MRSA)	1	1	*M. catarrhalis, S. pneumoniae*
M. catarrhalis	*S. aureus*	*S. pneumoniae*					-	2	2	*M. catarrhalis* (2), *S. aureus* (1), *S. pneumoniae* (1)
Proteus spp.	*P. aeruginosa*	*S. marcescens*					-	2	2	*Proteus* spp. (2)
Proteus spp.	*P. aeruginosa*	*S. aureus*					*mecA/C* & MREJ (MRSA)	1	1	*S. aureus*
Proteus spp.	*P. aeruginosa*	*S. pneumoniae*					-	1	1	*Proteus* spp.
P. aeruginosa	*S. marcescens*	*S. agalactiae*					KPC	1	1	*S. agalactiae*
P. aeruginosa	*S. aureus*	*S. agalactiae*					*mecA/C* & MREJ (MRSA)	2	2	*S. aureus* (1), *S. agalactiae* (1)
P. aeruginosa	*S. agalactiae*	*S. pneumoniae*					-	1	1	*S. agalactiae, S. pneumoniae*
ACB complex	*E. cloacae* complex						-	1	1	ACB complex
ACB complex	*P. aeruginosa*						-	1	1	ACB complex
ACB complex	*S. aureus*						*mecA/C* & MREJ (MRSA)	3	2	ACB complex (1), *S. aureus* (2), [*mecA/C* & MREJ (MRSA) (1)]
E. cloacae complex	*E. coli*						-	2	2	*E. cloacae* complex (1), *E. coli* (2)
E. cloacae complex	*K. oxytoca*						-	1	0	-

Distinct Co-Detection Combinations								Total Specimens with Co-Detection Combination	Number of Specimens with False Positive Co-Detections	False Positive Analyte(s) [False Positive AMR Gene(s)[a]]
Analyte 1	**Analyte 2**	**Analyte 3**	**Analyte 4**	**Analyte 5**	**Analyte 6**	**Analyte 7**	**AMR Gene(s)**			
E. cloacae complex	*K. pneumoniae* group						-	1	1	*E. cloacae* complex, *K. pneumoniae* group
E. cloacae complex	*P. aeruginosa*						-	1	1	*E. cloacae* complex, *P. aeruginosa*
E. cloacae complex	*S. aureus*						*mecA/C* & MREJ (MRSA)	3	1	*E. cloacae* complex (1)
E. coli	*H. influenzae*						-	1	1	*H. influenzae*
E. coli	*K. pneumoniae* group						KPC	1	0	-
E. coli	*K. pneumoniae* group						-	1	1	*K. pneumoniae* group
E. coli	*M. catarrhalis*						-	1	1	*E. coli, M. catarrhalis*
E. coli	*Proteus* spp.						CTX-M	1	1	*E. coli, Proteus* spp.
E. coli	*P. aeruginosa*						CTX-M	1	0	-
E. coli	*P. aeruginosa*						-	2	1	*E. coli* (1), *P. aeruginosa* (1)
E. coli	*S. aureus*						*mecA/C* & MREJ (MRSA)	3	3	*E. coli* (2), *S. aureus* (2)
E. coli	*S. aureus*						-	1	1	*S. aureus*
E. coli	*S. pyogenes*						-	1	1	*S. pyogenes*
H. influenzae	*M. catarrhalis*							6	6	*H. influenzae* (4), *M. catarrhalis* (6)
H. influenzae	*Proteus* spp.						-	1	1	*H. influenzae*
H. influenzae	*P. aeruginosa*						-	1	1	*H. influenzae*
H. influenzae	*S. marcescens*						-	1	1	*H. influenzae*
H. influenzae	*S. aureus*						*mecA/C* & MREJ (MRSA)	2	2	*H. influenzae* (2), *S. aureus* (2), [mecA/C & MREJ (MRSA) (1)]
H. influenzae	*S. aureus*						-	10	9	*H. influenzae* (9), *S. aureus* (6)
H. influenzae	*S. agalactiae*						-	1	1	*H. influenzae*
H. influenzae	*S. pneumoniae*						-	4	4	*H. influenzae* (4), *S. pneumoniae* (4)
K. aerogenes	*S. aureus*						-	3	3	*K. aerogenes (2), S. aureus (2)*

Distinct Co-Detection Combinations								Total Specimens with Co-Detection Combination	Number of Specimens with False Positive Co-Detections	False Positive Analyte(s) [False Positive AMR Gene(s)[a]]
Analyte 1	**Analyte 2**	**Analyte 3**	**Analyte 4**	**Analyte 5**	**Analyte 6**	**Analyte 7**	**AMR Gene(s)**			
K. oxytoca	*K. pneumoniae* group						-	2	0	-
K. oxytoca	*M. catarrhalis*						-	1	1	*K. oxytoca*, *M. catarrhalis*
K. oxytoca	*P. aeruginosa*						-	1	1	*K. oxytoca*
K. oxytoca	*S. aureus*						-	2	0	-
K. pneumoniae group	*P. aeruginosa*						KPC	1	1	*K. pneumoniae* group
K. pneumoniae group	*P. aeruginosa*						-	4	4	*K. pneumoniae* group (4), *P. aeruginosa* (2)
K. pneumoniae group	*S. aureus*						CTX-M, *mecA/C* & MREJ (MRSA)	1	1	*K. pneumoniae* group
K. pneumoniae group	*S. aureus*						*mecA/C* & MREJ (MRSA)	3	2	*K. pneumoniae* group (1), *S. aureus* (2), [*mecA/C* & MREJ (MRSA) (2)]
K. pneumoniae group	*S. agalactiae*						-	1	1	*K. pneumoniae* group, *S. agalactiae*
M. catarrhalis	*P. aeruginosa*						-	3	3	*M. catarrhalis* (2), *P. aeruginosa* (2)
M. catarrhalis	*S. marcescens*						-	2	2	*M. catarrhalis* (2)
M. catarrhalis	*S. aureus*						*mecA/C* & MREJ (MRSA)	4	3	*M. catarrhalis* (3), *S. aureus* (2)
M. catarrhalis	*S. aureus*						-	2	2	*M. catarrhalis* (1), *S. aureus* (1)
M. catarrhalis	*S. agalactiae*						-	1	1	*M. catarrhalis*
M. catarrhalis	*S. pneumoniae*						-	4	4	*M. catarrhalis* (4), *S. pneumoniae* (3)
Proteus spp.	*P. aeruginosa*						-	1	1	-
Proteus spp.	*S. aureus*						*mecA/C* & MREJ (MRSA)	1	0	-
P. aeruginosa	*S. marcescens*						-	7	4	*P. aeruginosa* (2), *S. marcescens* (2)
P. aeruginosa	*S. aureus*						CTX-M, *mecA/C* & MREJ (MRSA)	1	0	[CTX-M, *mecA/C* & MREJ (MRSA)]

Distinct Co-Detection Combinations								Total Specimens with Co-Detection Combination	Number of Specimens with False Positive Co-Detections	False Positive Analyte(s) [False Positive AMR Gene(s)[a]]
Analyte 1	**Analyte 2**	**Analyte 3**	**Analyte 4**	**Analyte 5**	**Analyte 6**	**Analyte 7**	**AMR Gene(s)**			
P. aeruginosa	*S. aureus*						*mecA/C* & MREJ (MRSA)	12	8	*P. aeruginosa* (4), *S. aureus* (8)
P. aeruginosa	*S. aureus*						-	4	2	*P. aeruginosa* (2), *S. aureus* (1)
P. aeruginosa	*S. pneumoniae*						-	1	1	*P. aeruginosa, S. pneumoniae*
S. marcescens	*S. aureus*						*mecA/C* & MREJ (MRSA)	3	2	*S. marcescens* (2), *S. aureus* (1), [*mecA/C* & MREJ (MRSA) (1)]
S. marcescens	*S. aureus*						-	2	2	*S. marcescens* (1), *S. aureus* (1)
S. marcescens	*S. pyogenes*						VIM	1	1	*S. marcescens*[b], *S. pyogenes,* [VIM]
S. aureus	*S. agalactiae*						*mecA/C* & MREJ (MRSA)	4	4	*S. aureus* (2), *S. agalactiae* (4)
S. aureus	*S. agalactiae*						-	4	3	*S. aureus* (2), *S. agalactiae* (3)
S. aureus	*S. pneumoniae*						*mecA/C* & MREJ (MRSA)	1	1	*S. pneumoniae,* [*mecA/C* & MREJ (MRSA)]
S. aureus	*S. pneumoniae*							5	4	*S. aureus* (2), *S. pneumoniae* (4)
S. aureus	*S. pyogenes*						*mecA/C* & MREJ (MRSA)	1	0	-
Total Co-Detections								**246**	**209**	**392[b]/667**
Total Double Detections								**135**	**106**	**155/270**
Total Triple Detections								**71**	**65**	**125/213**
Total Quadruple Detections								**23**	**21**	**52/92**
Total Quintuple Detections								**12**	**12**	**40/60**
Total Sextuple Detections								**3**	**3**	**11/18**
Total Septuple Detections								**2**	**2**	**9/14**

[a] AMR genes compared to qMol.

[b] Discrepant analyte could not be confirmed as being present in the specimen during discrepancy investigation.

[c] Of the 392 discrepant analytes (out of 667 total analytes), 390 (99.5%) were observed as being present in the specimen during discrepancy investigation; 79/392 (20.2%) were enumerated below 10^3.5 CFU/mL by qRefCx, 271/392 (69.1%) were detected by qMol, 34/392 (8.7%) were detected using an additional molecular method, and 6/392 (1.5%) were identified in SOC culture.

PERFORMANCE CHARACTERISTICS

NOTE: BIOFIRE Pneumonia Panel performance was initially established on BIOFIRE® FILMARRAY® (BIOFIRE), BIOFIRE 2.0, and BIOFIRE TORCH systems. The BIOFIRE system (REF: FLM1-ASY-0001) is no longer being manufactured or distributed, but the performance characteristics established on that system are relevant to the BIOFIRE Pneumonia Panel and remain in this Instructions for Use. Comparison studies have established that BioFire Pneumonia Panel performance characteristics are equivalent between BIOFIRE, BIOFIRE 2.0, and BIOFIRE TORCH systems.

Clinical Performance

The clinical performance of the BIOFIRE Pneumonia Panel was established during a multi-center study conducted at eight geographically distinct U.S. study sites from October 2016 to July 2017. A total of 904 residual BAL (821 BAL and 83 mini-BAL) and 925 residual sputum (478 sputum and 447 ETA) specimens were acquired for the prospective clinical study. BIOFIRE Pneumonia Panel performance in BAL and mini-BAL was similar, as was performance in sputum and ETA; therefore, these sample types are not stratified further in performance tables. A total of 58 BAL and 89 sputum specimens were excluded from the final data analysis. The most common reasons for specimen exclusion for both specimen types was reference culture unable to be performed, the specimen was found to not meet the inclusion criteria after the specimen had been enrolled, or the study site was unable to complete the Case Report Form (CRF). The final data set consisted of 846 BAL and 836 sputum specimens. Table 20 and Table 21 provides a summary of demographic information for the specimens included in the prospective study.

Table 20. Overall and Per Site Demographic Analysis for BAL Specimens

BAL										
		Overall	**Site 1[a]**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**	**Site 7**	**Site 8**
Sex	Male	480 (57%)	80 (59%)	7 (54%)	138 (55%)	21 (68%)	75 (61%)	82 (52%)	27 (61%)	50 (55%)
	Female	366 (43%)	55 (41%)	6 (46%)	113 (45%)	10 (32%)	48 (39%)	76 (48%)	17 (39%)	41 (45%)
Age	≤ 5 years	23 (3%)	0 (0%)	5 (38%)	0 (0%)	15 (48%)	0 (0%)	3 (2%)	0 (0%)	0 (0%)
	6 - 17 years	27 (3%)	0 (0%)	8 (62%)	0 (0%)	13 (42%)	0 (0%)	4 (3%)	1 (2%)	1 (1%)
	18 - 34 years	70 (8%)	18 (13%)	0 (0%)	17 (7%)	3 (10%)	10 (8%)	10 (6%)	5 (11%)	7 (8%)
	35 - 65 years	470 (56%)	78 (58%)	0 (0%)	152 (61%)	0 (0%)	70 (57%)	88 (56%)	27 (61%)	55 (60%)
	> 65 years	255 (30%)	38 (28%)	0 (0%)	82 (33%)	0 (0%)	43 (35%)	53 (34%)	11 (25%)	28 (31%)
Care Setting	Hospitalized	666 (79%)	116 (86%)	12 (92%)	223 (89%)	9 (29%)	82 (67%)	118 (75%)	25 (57%)	81 (89%)
	Outpatient	159 (19%)	18 (13%)	0 (0%)	28 (11%)	22 (71%)	31 (25%)	39 (25%)	14 (32%)	7 (8%)
	Emergency	21 (2%)	1 (1%)	1 (8%)	0 (0%)	0 (0%)	10 (8%)	1 (1%)	5 (11%)	3 (3%)
Total		**846**	**135**	**13**	**251**	**31**	**123**	**158**	**44**	**91**

[a] Subject age could not be determined for one specimen from Site 1.

Table 21. Overall and Per Site Demographic Analysis for Sputum Specimens

Sputum										
		Overall	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**	**Site 7**	**Site 8**
Sex	Male	481 (58%)	66 (59%)	54 (54%)	136 (56%)	97 (61%)	14 (82%)	31 (53%)	34 (74%)	49 (47%)
	Female	355 (42%)	45 (41%)	46 (46%)	105 (44%)	61 (39%)	3 (18%)	28 (47%)	12 (26%)	55 (53%)
Age	≤ 5 years	138 (17%)	0 (0%)	49 (49%)	0 (0%)	80 (51%)	0 (0%)	0 (0%)	2 (4%)	7 (7%)

Sputum										
		Overall	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**	**Site 7**	**Site 8**
	6 - 17 years	107 (13%)	0 (0%)	35 (35%)	0 (0%)	64 (41%)	0 (0%)	0 (0%)	2 (4%)	6 (6%)
	18 - 34 years	86 (10%)	15 (14%)	16 (16%)	20 (8%)	13 (8%)	1 (6%)	6 (10%)	5 (11%)	10 (10%)
	35 - 65 years	284 (34%)	51 (46%)	0 (0%)	133 (55%)	1 (1%)	6 (35%)	36 (61%)	20 (43%)	37 (36%)
	> 65 years	221 (26%)	45 (41%)	0 (0%)	88 (37%)	0 (0%)	10 (59%)	17 (29%)	17 (37%)	44 (42%)
Care Setting	Hospitalized	682 (82%)	106 (95%)	64 (64%)	219 (91%)	105 (66%)	12 (71%)	52 (88%)	23 (50%)	101 (97%)
	Outpatient	73 (9%)	2 (2%)	14 (14%)	18 (7%)	24 (15%)	2 (12%)	5 (8%)	7 (15%)	1 (1%)
	Emergency	81 (10%)	3 (3%)	22 (22%)	4 (2%)	29 (18%)	3 (18%)	2 (3%)	16 (35%)	2 (2%)
Total		**836**	**111**	**100**	**241**	**158**	**17**	**59**	**46**	**104**

All specimens were evaluated with the BIOFIRE Pneumonia Panel at clinical study sites. Refrigerated specimen aliquots were sent to a central reference laboratory for quantitative reference culture (qRefCx) and frozen specimen aliquots were also sent to BIOFIRE for evaluation by polymerase chain reaction (PCR)/sequencing-based comparator methods.

The reference methods used in this study were as follows:

Bacterial analytes were compared to qRefCx to evaluate sensitivity and specificity, and the method was considered positive for the presence of the organism of interest if it was recovered in culture and enumerated at a level of 3162 (10^3.5) CFU/mL or greater.

Bacterial analytes were also evaluated by comparison to a single PCR assay for the organism of interest followed by a quantitative molecular assay that included sequencing (qMol) to assess BIOFIRE bin reporting performance. Atypical bacteria and viruses were compared to two conventional PCR assays followed by bidirectional sequencing. For specimens with an applicable bacteria detected by BIOFIRE, AMR genes were compared to a single PCR assay (from the specimen) followed by sequencing. A specimen was considered to be positive for an analyte if bi-directional sequencing data meeting pre-defined quality acceptance criteria matched organism-specific sequences deposited in the NCBI GenBank database (www.ncbi.nlm.nih.gov) with acceptable E-values. When two PCR comparator assays were used, any specimen that tested negative by both of the comparator assays was considered Negative.

Positive Percent Agreement (PPA) or Sensitivity for each analyte was calculated as 100% x (TP / (TP + FN)). True positive (TP) indicates that both the BIOFIRE Pneumonia Panel and the comparator method had a positive result for this specific analyte, and false negative (FN) indicates that the BIOFIRE Pneumonia Panel result was negative while the comparator result was positive. Negative Percent Agreement (NPA) or Specificity was calculated as 100% x (TN / (TN + FP)). True negative (TN) indicates that both the BIOFIRE Pneumonia Panel and the comparator method had negative results, and a false positive (FP) indicates that the BIOFIRE Pneumonia Panel result was positive but the comparator result was negative. The exact binomial two-sided 95% confidence interval was calculated. Samples for which false positive and/or false negative results (i.e., discrepant results) were obtained when comparing the BIOFIRE Pneumonia Panel results to the comparator method results were further investigated. The discrepancy investigations were primarily performed as follows: for discrepancies between the BIOFIRE Pneumonia Panel and reference culture for bacterial analytes, discrepancies were first examined to see if qRefCx or BIOFIRE had observed the analyte but reported it as “negative” or “Not Detected” because it was below the detection threshold. If this did not resolve the discrepancy, the results of qMol testing were considered. And if these methods still did not resolve the discrepancy, it was then investigated in the same manner as other analytes that used molecular comparator (i.e. using multiple additional molecular assays followed by sequence analysis). The results of SOC testing were also considered. The prospective clinical study results are summarized in Table 22 and Table 23 for BAL and sputum specimens, respectively.

Table 22. BIOFIRE Pneumonia Panel Clinical Performance Summary for BAL Specimens[a]

BAL							
Analyte	Reference Method	Sensitivity/PPA			Specificity/NPA		
		TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Bacteria							
***Acinetobacter calcoaceticus-baumannii* complex[b]**	qRefCx	0/0	-	-	839/846	99.2	98.3-99.6%
***Enterobacter cloacae* complex[c]**	qRefCx	11/12	91.7	64.6-98.5%	822/834	98.6	97.5-99.2%
***Escherichia coli*[d]**	qRefCx	12/12	100	75.8-100%	826/834	99.0	98.1-99.5%
***Haemophilus influenzae*[e]**	qRefCx	10/10	100	72.2-100%	764/836	91.4	89.3-93.1%
***Klebsiella aerogenes*[f]**	qRefCx	6/7	85.7	48.7-97.4%	832/839	99.2	98.3-99.6%
***Klebsiella oxytoca*[g]**	qRefCx	2/2	100	34.2-100%	835/844	98.9	98.0-99.4%
***Klebsiella pneumoniae* group[h]**	qRefCx	15/15	100	79.6-100%	819/831	98.6	97.5-99.2%
***Moraxella catarrhalis*[i]**	qRefCx	0/0	-	-	817/846	96.6	95.1-97.6%
***Proteus* spp.[j]**	qRefCx	5/5	100	56.6-100%	837/841	99.5	98.8-99.8%
***Pseudomonas aeruginosa*[k]**	qRefCx	36/36	100	90.4-100%	772/810	95.3	93.6-96.6%
***Serratia marcescens*[l]**	qRefCx	6/6	100	61.0-100%	834/840	99.3	98.5-99.7%
***Staphylococcus aureus*[m]**	qRefCx	46/47	97.9	88.9-99.6%	729/799	91.2	89.1-93.0%
***Streptococcus agalactiae*[n]**	qRefCx	1/1	100	-	821/845	97.2	95.8-98.1%
***Streptococcus pneumoniae*[o]**	qRefCx	5/5	100	56.6-100%	817/841	97.1	95.8-98.1%
***Streptococcus pyogenes*[p]**	qRefCx	2/2	100	34.2-100%	838/844	99.3	98.5-99.7%
Atypical Bacteria							
***Chlamydia pneumoniae*[q]**	PCR/Seq	0/0	-	-	844/845	99.9	99.3-100%
Legionella pneumophila	PCR/Seq	2/2	100	34.2-100%	833/833	100	99.5-100%
***Mycoplasma pneumoniae*[r]**	PCR/Seq	3/3	100	43.9-100%	841/842	99.9	99.3-100%
Viruses							
Adenovirus	PCR/Seq	8/8	100	67.6-100%	837/837	100	99.5-100%
Coronavirus[s]	PCR/Seq	18/21	85.7	65.4-95.0%	810/823	98.4	97.3-99.1%
Human metapneumovirus[t]	PCR/Seq	8/8	100	67.6-100%	836/837	99.9	99.3-100%
Human rhinovirus/enterovirus[u]	PCR/Seq	52/54	96.3	87.5-99.0%	771/782	98.6	97.5-99.2%
Influenza A virus[v]	PCR/Seq	10/10	100	72.2-100%	830/833	99.6	98.9-99.9%
Influenza B virus[w]	PCR/Seq	5/6	83.3	43.6-97.0%	837/838	99.9	99.3-100%
Parainfluenza virus[x]	PCR/Seq	16/18	88.9	67.2-96.9%	824/826	99.8	99.1-99.9%
Respiratory syncytial virus	PCR/Seq	3/3	100	43.9-100%	841/841	100	99.5-100%

[a] The performance measures of sensitivity and specificity only refer to the bacterial analytes for which the gold-standard of qRefCx was used as the reference method. Performance measures of PPA and NPA refer to all other analytes, for which PCR/sequencing assays were used as comparator methods.

[b] Evidence of ACB complex was found in all seven FP specimens; one was enumerated below 10^3.5 CFU/mL by qRefCx and six were detected by qMol.

[c] *E. cloacae* complex was observed in the single FN specimen below the 10^4 bin by the BIOFIRE Pneumonia Panel. Evidence of *E. cloacae* complex was found in all 12 FP specimens; six were enumerated below 10^3.5 CFU/mL by qRefCx, five were detected by qMol, and one was detected using an additional molecular method.

[d] Evidence of *E. coli* was found in all eight FP specimens; six were enumerated below 10^3.5 CFU/mL by qRefCx and two were detected by qMol.

[e] Evidence of *H. influenzae* was found in all 72 FP specimens; seven were enumerated below 10^3.5 CFU/mL by qRefCx, 56 were detected by qMol, eight were detected using an additional molecular method, and one was identified in SOC culture.

[f] *K. aerogenes* was identified in the single FN specimen in SOC culture. Evidence of *K. aerogenes* was found in all seven FP specimens; four were enumerated below 10^3.5 CFU/mL by qRefCx and three were detected by qMol.

[g] Evidence of *K. oxytoca* was found in all nine FP specimens; three were enumerated below 10^3.5 CFU/mL by qRefCx, five were detected by qMol, and one was detected using an additional molecular method.

[h] Evidence of *K. pneumoniae* group was found in all 12 FP specimens; seven were enumerated below 10^3.5 CFU/mL by qRefCx, four were detected by qMol, and one was detected using an additional molecular method.

[i] Evidence of *M. catarrhalis* was found in all 29 FP specimens; two were enumerated below 10^3.5 CFU/mL by qRefCx, 25 were detected by qMol, and two were detected using an additional molecular method.

[j] Evidence of *Proteus* spp. was found in all four FP specimens; three were enumerated below 10^3.5 CFU/mL by qRefCx and one was detected by qMol.

[k] Evidence of *P. aeruginosa* was found in all 38 FP specimens; 19 were enumerated below 10^3.5 CFU/mL by qRefCx, 16 were detected by qMol, and three were detected using an additional molecular method.

[l] Evidence of *S. marcescens* was found in all six FP specimens; four were enumerated below 10^3.5 CFU/mL by qRefCx and two were detected by qMol.

[m] *S. aureus* was detected in the single FN specimen using an additional molecular method. Evidence of *S. aureus* was found in 69/70 FP specimens; 29 were enumerated below 10^3.5 CFU/mL by qRefCx, 30 were detected by qMol, eight were detected using an additional molecular method, and two were identified in SOC culture.

[n] Evidence of *S. agalactiae* was found in all 24 FP specimens; seven were enumerated below 10^3.5 CFU/mL by qRefCx, 13 were detected by qMol, and four were detected using an additional molecular method.

[o] Evidence of *S. pneumoniae* was found in all 24 FP specimens; five were enumerated below 10^3.5 CFU/mL by qRefCx, 18 were detected by qMol, and one was detected using an additional molecular method.

[p] Evidence of *S. pyogenes* was found in all six FP specimens; two were enumerated below 10^3.5 CFU/mL by qRefCx, three were detected by qMol, and one was detected using an additional molecular method.

[q] The single FP specimen was negative for *C. pneumoniae* when tested with additional molecular methods during discrepancy investigation.

[r] The single FP specimen was negative for *M. pneumoniae* when tested with additional molecular methods during discrepancy investigation.

[s] CoV was detected in 2/3 FN and 8/13 FP specimens using an additional molecular method.

[t] The single FP specimen was negative for hMPV when tested with additional molecular methods during discrepancy investigation.

[u] HRV/EV was detected in both FN specimens using an additional molecular method. HRV/EV was detected in 8/11 FP specimens during discrepancy investigation; seven were detected using an additional molecular method and one was detected upon BIOFIRE Pneumonia Panel retest.

[v] FluA was detected in 2/3 FP specimens using an additional molecular method.

[w] FluB was detected in the single FN specimen upon BIOFIRE Pneumonia Panel retest. FluB was detected in the single FP specimen using an additional molecular method.

[x] PIV was detected in both FN and both FP specimens using an additional molecular method.

Table 23. BIOFIRE Pneumonia Panel Clinical Performance Summary for Sputum Specimens[a]

Sputum							
Analyte	**Reference Method**	**Sensitivity/PPA**			**Specificity/NPA**		
		TP/(TP + FN)	**%**	**95%CI**	**TN/(TN + FP)**	**%**	**95%CI**
Bacteria							
***Acinetobacter calcoaceticus-baumannii* complex[b]**	qRefCx	10/11	90.9	62.3-98.4%	807/825	97.8	96.6-98.6%
***Enterobacter cloacae* complex[c]**	qRefCx	11/12	91.7	64.6-98.5%	803/824	97.5	96.1-98.3%
***Escherichia coli*[d]**	qRefCx	23/24	95.8	79.8-99.3%	787/812	96.9	95.5-97.9%
***Haemophilus influenzae*[e]**	qRefCx	16/18	88.9	67.2-96.9%	727/818	88.9	86.5-90.9%
***Klebsiella aerogenes*[f]**	qRefCx	3/4	75.0	30.1-95.4%	823/832	98.9	98.0-99.4%
***Klebsiella oxytoca*[g]**	qRefCx	9/9	100	70.1-100%	817/827	98.8	97.8-99.3%
***Klebsiella pneumoniae* group[h]**	qRefCx	21/23	91.3	73.2-97.6%	769/813	94.6	92.8-95.9%
***Moraxella catarrhalis*[i]**	qRefCx	5/5	100	56.6-100%	761/831	91.6	89.5-93.3%
***Proteus* spp.[j]**	qRefCx	15/15	100	79.6-100%	813/821	99.0	98.1-99.5%
***Pseudomonas aeruginosa*[k]**	qRefCx	103/106	97.2	92.0-99.0%	673/730	92.2	90.0-93.9%
***Serratia marcescens*[l]**	qRefCx	26/27	96.3	81.7-99.3%	782/809	96.7	95.2-97.7%
***Staphylococcus aureus*[m]**	qRefCx	111/112	99.1	95.1-99.8%	631/724	87.2	84.5-89.4%
***Streptococcus agalactiae*[n]**	qRefCx	9/9	100	70.1-100%	793/827	95.9	94.3-97.0%
***Streptococcus pneumoniae*[o]**	qRefCx	16/16	100	80.6-100%	785/820	95.7	94.1-96.9%
***Streptococcus pyogenes*[p]**	qRefCx	6/6	100	61.0-100%	825/830	99.4	98.6-99.7%
Atypical Bacteria							
Chlamydia pneumoniae	PCR/Seq	0/0	-	-	835/835	100	99.5-100%
***Legionella pneumophila*[q]**	PCR/Seq	0/1	0	-	826/826	100	99.5-100%
***Mycoplasma pneumoniae*[r]**	PCR/Seq	7/8	87.5	52.9-97.8%	827/827	100	99.5-100%
Viruses							
Adenovirus[s]	PCR/Seq	13/17	76.5	52.7-90.4%	815/817	99.8	99.1-99.9%
Coronavirus[t]	PCR/Seq	28/32	87.5	71.9-95.0%	796/802	99.3	98.4-99.7%
Human metapneumovirus[u]	PCR/Seq	20/21	95.2	77.3-99.2%	812/813	99.9	99.3-100%
Human rhinovirus/enterovirus[v]	PCR/Seq	96/96	100	96.2-100%	717/730	98.2	97.0-99.0%
Influenza A virus[w]	PCR/Seq	13/13	100	77.2-100%	819/822	99.6	98.9-99.9%

Sputum							
Analyte	Reference Method	Sensitivity/PPA			Specificity/NPA		
		TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Influenza B virus[x]	PCR/Seq	12/12	100	75.8-100%	821/823	99.8	99.1-99.9%
Parainfluenza virus[y]	PCR/Seq	28/29	96.6	82.8-99.4%	804/806	99.8	99.1-99.9%
Respiratory Syncytial virus[z]	PCR/Seq	43/43	100	91.8-100%	787/791	99.5	98.7-99.8%

[a] The performance measures of sensitivity and specificity only refer to the bacterial analytes for which the gold-standard of qRefCx was used as the reference method. Performance measures of PPA and NPA refer to all other analytes, for which PCR/sequencing assays were used as comparator methods.

[b] The isolate recovered from the single FN specimen was misidentified by qRefCx; molecular testing of the isolate identified it as *Pseudomonas fluorescens* during discrepancy investigation. Evidence of ACB complex was found in all 18 FP specimens; 15 were detected by qMol, two were detected using an additional molecular method, and one was identified in SOC culture.

[c] *E. cloacae* complex was detected in the single FN specimen using an additional molecular method. Evidence of *E. cloacae* complex was found in all 21 FP specimens; four were enumerated below 10^3.5 CFU/mL by qRefCx, 16 were detected by qMol, and one was detected using an additional molecular method.

[d] *E. coli* was observed in the single FN specimen below the 10^4 bin by the BIOFIRE Pneumonia Panel. Evidence of *E. coli* was found in all 25 FP specimens; six were enumerated below 10^3.5 CFU/mL by qRefCx, 14 were detected by qMol, and five were detected using an additional molecular method.

[e] *H. influenzae* was detected in 1/2 FN specimens by qMol. The isolate recovered from the other FN specimen was misidentified by qRefCx; molecular testing of the isolate identified it as *Haemophilus haemolyticus* during discrepancy investigation. Evidence of *H. influenzae* was found in all 91 FP specimens; four were enumerated below 10^3.5 CFU/mL by qRefCx, 78 were detected by qMol, seven were detected using an additional molecular method, and two were identified in SOC culture.

[f] The isolate recovered from the single FN specimen was misidentified by qRefCx; molecular testing of the isolate identified it as *Hafnia paralvei* during discrepancy investigation. Evidence of *K. aerogenes* was found in all nine FP specimens; three were enumerated below 10^3.5 CFU/mL by qRefCx, five were detected by qMol, and one was detected using an additional molecular method.

[g] Evidence of *K. oxytoca* was found in all 10 FP specimens; three were enumerated below 10^3.5 CFU/mL by qRefCx, five were detected by qMol, and two were detected using an additional molecular method.

[h] *K. pneumoniae* group was detected in 1/2 FN specimens by qMol. The other FN appeared to be a result of a specimen swap at the central reference laboratory. Evidence of *K. pneumoniae* group was found in 43/44 FP specimens; 15 were enumerated below 10^3.5 CFU/mL by qRefCx, 21 were detected by qMol, and seven were detected using an additional molecular method.

[i] Evidence of *M. catarrhalis* was found in all 70 FP specimens; one was enumerated below 10^3.5 CFU/mL by qRefCx, 63 were detected by qMol, five were detected using an additional molecular method, and one was identified in SOC culture.

[j] Evidence of *Proteus* spp. was found in all eight FP specimens; two were enumerated below 10^3.5 CFU/mL by qRefCx, four were detected by qMol, and two were detected using an additional molecular method.

[k] *P. aeruginosa* was observed in 1/3 FN specimens below the 10^4 bin by the BIOFIRE Pneumonia Panel. The isolates recovered from the other two FN specimens were misidentified by qRefCx; molecular testing of the isolates identified one as *Pseudomonas denitrificans* and the other as *Pseudomonas fluorescens* during discrepancy investigation. Evidence of *P. aeruginosa* was found in all 57 FP specimens; 21 were enumerated below 10^3.5 CFU/mL by qRefCx, 33 were detected by qMol, two were detected using an additional molecular method, and one was identified in SOC culture.

[l] *S. marcescens* was observed in the single FN specimen below the 10^4 bin by the BIOFIRE Pneumonia Panel. Evidence of *S. marcescens* was found in 26/27 FP specimens; seven were enumerated below 10^3.5 CFU/mL by qRefCx, 16 were detected by qMol, and three were detected using an additional molecular method.

[m] *S. aureus* was observed in the single FN specimen below the 10^4 bin by the BIOFIRE Pneumonia Panel. Evidence of *S. aureus* was found in all 93 FP specimens; 43 were enumerated below 10^3.5 CFU/mL by qRefCx, 43 were detected by qMol, three were detected using an additional molecular method, and four were identified in SOC culture.

[n] Evidence of *S. agalactiae* was found in all 34 FP specimens; five were enumerated below 10^3.5 CFU/mL by qRefCx, 24 were detected by qMol, and five were detected using an additional molecular method.

[o] Evidence of *S. pneumoniae* was found in all 35 FP specimens; one was enumerated below 10^3.5 CFU/mL by qRefCx and 34 were detected by qMol.

[p] Evidence of *S. pyogenes* was found in all five FP specimens; four were detected by qMol and one was detected using an additional molecular method.

[q] *L. pneumophila* was detected in the single FN specimen using an additional molecular method.

[r] The single FN specimen was negative for *M. pneumoniae* when tested with an additional molecular method during discrepancy investigation.

[s] AdV was detected in all four FN and 1/2 FP specimens using an additional molecular method.

[t] CoV was detected in all four FN and 3/6 FP specimens using an additional molecular method.

[u] hMPV was detected in the single FN specimen using an additional molecular method. The single FP specimen was negative for hMPV when tested with an additional molecular method during discrepancy investigation.

[v] HRV/EV was detected in 12/13 FP specimens during discrepancy investigation; 11 were detected using an additional molecular method and one was detected upon BIOFIRE Pneumonia Panel retest.

[w] FluA was detected in all three FP specimens using an additional molecular method.

[x] Both FP specimens were negative for FluB when tested with additional molecular methods during discrepancy investigation.

[y] PIV was detected in the single FN and 1/2 FP specimens using an additional molecular method.

[z] RSV was detected in all four FP specimens using an additional molecular method.

A total of 156 BAL specimens and 295 sputum specimens received a BIOFIRE Pneumonia Panel Detected result for at least one applicable gram-negative bacterium on the panel and reported results for CTX-M, IMP, KPC, NDM, and VIM; a total of 94 BAL specimens and 196 sputum specimens received a BIOFIRE Pneumonia Panel Detected result for at least one applicable gram-negative bacterium on the panel and reported results for OXA-48-like; and a total of 116 BAL specimens and 204 sputum specimens received a BIOFIRE Pneumonia Panel *Staphylococcus aureus* Detected result and reported results for *mecA/C* and MREJ (MRSA). Performance of the BIOFIRE Pneumonia Panel AMR gene assays was calculated by comparing results of qMol direct from these specimens and is shown in Table 24 (five BAL and four sputum specimens were excluded from qMol analysis due to invalid comparator results).

Table 24. BIOFIRE Pneumonia Panel Clinical Performance Summary – AMR Genes (comparator method: qMol direct from specimen)[a]

Analyte	BAL						Sputum					
	PPA			NPA			PPA			NPA		
	TP/ (TP + FN)	%	95%CI	TN/ (TN + FP)	%	95%CI	TP/ (TP + FN)	%	95%CI	TN/ (TN + FP)	%	95%CI
CTX-M[b]	6/7	85.7	48.7-97.4%	144/144	100	97.4-100%	8/10	80.0	49.0-94.3%	280/281	99.6	98.0-99.9%
IMP	0/0	-	-	151/151	100	97.5-100%	0/0	-	-	291/291	100	98.7-100%
KPC[c]	2/2	100	34.2-100%	148/149	99.3	96.3-99.9%	7/7	100	64.6-100%	284/284	100	98.7-100%
***mecA/C* and MREJ[d] (MRSA)**	40/45	88.9	76.5-95.2%	64/70	91.4	82.5-96.0%	94/98	95.9	90.0-98.4%	91/104	87.5	79.8-92.5%
NDM[e]	0/1	0	-	149/150	99.3	96.3-99.9%	0/0	-	-	291/291	100	98.7-100%
OXA-48-like	0/0	-	-	92/92	100	96.0-100%	0/0	-	-	195/195	100	98.1-100%
VIM[f]	0/0	-	-	151/151	100	97.5-100%	1/1	100	-	289/290	99.7	98.1-99.9%

[a] Performance in this summary table is calculated when *any* applicable organism is detected in the sample.

[b] CTX-M was detected in the single FN BAL and 1/2 FN sputum specimens using an additional molecular method. The single FP sputum specimen was negative for CTX-M when tested during discrepancy investigation. None of the applicable isolates identified by the BIOFIRE or qRefCx from these specimens had evidence of ESBL activity or CTX-M presence.

[c] KPC was detected in the single FP BAL specimen using an additional molecular method; the isolate recovered from this specimen (*A. baumannii*) exhibited carbapenem resistance but did not carry KPC.

[d] Evidence of *mecA/C* and/or SCC*mec* cassette genetic elements was found in all five FN BAL and all four FN sputum specimens by an additional molecular method; three of these also had a MRSA isolate recovered via qRefCx or SOC culture. Evidence of *mecA/C* and/or SCC*mec* cassette genetic elements was found in 5/6 FP BAL and all 13 FP sputum specimens; nine had a MRSA isolate recovered via qRefCx or SOC culture, and nine additional specimens had evidence of *mecA/C* and/or SCC*mec* cassette genetic elements by an additional molecular method.

[e] NDM was detected in the single FN BAL specimen using an additional molecular method; *P. aeruginosa* was recovered from the specimen and was resistant to carbapenems but carried only KPC. The single FP BAL specimen was negative for NDM when tested with additional molecular methods during discrepancy investigation.

[f] The single FP sputum specimen was negative for VIM when tested with additional molecular methods during discrepancy investigation.

qRefCx isolated one or more applicable gram-negative bacteria from 127 of the 156 BAL specimens and 230 of the 295 sputum specimens that received a BIOFIRE Pneumonia Panel Detected result for an applicable gram-negative bacterium for CTX-M, IMP, KPC, NDM, and VIM. The method used to assess correlation of the CTX-M, IMP, KPC, NDM, and VIM results (Table 25 and Table 26) reported in the specimen by the BIOFIRE Pneumonia Panel to identification of the gene in the cultured isolates from that particular specimen was one conventional PCR assay followed by bidirectional sequencing, performed directly on the isolate.

Table 25. CTX-M, IMP, KPC, NDM, and VIM Performance Table (PCR/seq on cultured isolate(s) from BAL specimens)

BAL													
Applicable Bacteria Result (BIOFIRE)	**N**	**CTX-M**		**IMP**		**KPC**		**NDM**		**VIM**		***Overall*** (any resistance gene)	
		PPA	NPA	PPA	NPA	PPA	NPA	PPA	NPA	PPA	NPA	PPA	NPA
Overall *(any applicable bacteria Detected)*[a]	**127**	**4/4 (100%)**	**121/123 (98.4%)**	**0/0 (-)**	**127/127 (100%)**	**1/1 (100%)**	**124/126 (98.4%)**	**0/0 (-)**	**127/127 (100%)**	**0/0 (-)**	**127/127 (100%)**	**5/5 (100%) [56.6-100%]**	**118/122 (96.7%) [91.9-98.7%]**
Acinetobacter calcoaceticus-baumannii complex	0	-	-	-	-	-	-	-	-	-	-	-	-
Enterobacter cloacae complex	9	0/0 (-)	9/9 (100%)	0/0 (-)	9/9 (100%)	0/0 (-)	9/9 (100%)	0/0 (-)	9/9 (100%)	0/0 (-)	9/9 (100%)	0/0 (-)	9/9 (100%)
Escherichia coli	12	4/4 (100%)	8/8 (100%)	0/0 (-)	12/12 (100%)	0/0 (-)	12/12 (100%)	0/0 (-)	12/12 (100%)	0/0 (-)	12/12 (100%)	4/4 (100%)	8/8 (100%)
Klebsiella aerogenes	7	0/0 (-)	7/7 (100%)	0/0 (-)	7/7 (100%)	0/0 (-)	7/7 (100%)	0/0 (-)	7/7 (100%)	0/0 (-)	7/7 (100%)	0/0 (-)	7/7 (100%)
Klebsiella oxytoca	2	0/0 (-)	2/2 (100%)	0/0 (-)	2/2 (100%)	0/0 (-)	2/2 (100%)	0/0 (-)	2/2 (100%)	0/0 (-)	2/2 (100%)	0/0 (-)	2/2 (100%)
Klebsiella pneumoniae group	14	0/0 (-)	14/14 (100%)	0/0 (-)	14/14 (100%)	0/0 (-)	14/14 (100%)	0/0 (-)	14/14 (100%)	0/0 (-)	14/14 (100%)	0/0 (-)	14/14 (100%)
Proteus spp.	6	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)
Pseudomonas aeruginosa	43	0/0 (-)	42/43 (97.7%)	0/0 (-)	43/43 (100%)	0/0 (-)	43/43 (100%)	0/0 (-)	43/43 (100%)	0/0 (-)	43/43 (100%)	0/0 (-)	42/43 (97.7%)
Serratia marcescens	6	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)
Polymicrobial specimens	28	0/0 (-)	27/28[b] (96.4%)	0/0 (-)	28/28 (100%)	1/1[c] (100%)	25/27[d] (92.6%)	0/0 (-)	28/28 (100%)	0/0 (-)	28/28 (100%)	1/1 (100%)	24/27 (88.9%)

[a] An additional nine specimens had no applicable bacteria detected by BIOFIRE, but had one or more applicable bacteria isolated by qRefCx; no resistance markers were identified in the cultured isolate(s) by PCR/seq from these specimens.

[b] One specimen *E. cloacae* complex and *K. pneumoniae* group detected by BIOFIRE (*E. cloacae* complex isolated by qRefCx; CTX-M was not identified in this isolate by PCR/seq).

[c] *E. cloacae* complex and *P. aeruginosa* detected by BIOFIRE and isolated by qRefCx (KPC identified from the *E. cloacae* isolate by PCR/seq).

[d] One specimen *A. calcoaceticus-baumannii* complex and *K. pneumoniae* group detected by BIOFIRE (*A. calcoaceticus-baumannii* complex isolated by qRefCx; KPC was not identified in this isolate by PCR/seq); one specimen *Proteus* spp. and *P. aeruginosa* detected by BIOFIRE (*P. aeruginosa* isolated by qRefCx; KPC was not identified in this isolated by PCR/seq).

Table 26. CTX-M, IMP, KPC, NDM, and VIM Performance Table (PCR/seq on cultured isolate(s) from sputum specimens)

Sputum													
Applicable Bacteria Result	**N**	**CTX-M**		**IMP**		**KPC**		**NDM**		**VIM**		***Overall* (any resistance gene)**	
		PPA	**NPA**	**PPA**	**NPA**	**PPA**	**NPA**	**PPA**	**NPA**	**PPA**	**NPA**	**PPA**	**NPA**
Overall *(any applicable bacteria Detected)*[a]	**230**	**3/4 (75.0%)**	**221/226 (97.8%)**	**1/1 (100%)**	**229/229 (100%)**	**5/6 (83.3%)**	**223/224 (99.6%)**	**0/0 (-)**	**230/230 (100%)**	**1/1 (100%)**	**229/229 (100%)**	**9/11[b] (81.8%) [52.3-94.9%]**	**214/219 (97.7%) [94.8-99.0%]**
Acinetobacter calcoaceticus-baumannii complex	5[c]	0/0 (-)	5/5 (100%)	0/0 (-)	5/5 (100%)	0/0 (-)	5/5 (100%)	0/0 (-)	5/5 (100%)	0/0 (-)	5/5 (100%)	0/0 (-)	5/5 (100%)
Enterobacter cloacae complex	7[d]	0/0 (-)	7/7 (100%)	0/0 (-)	7/7 (100%)	0/0 (-)	7/7 (100%)	0/0 (-)	7/7 (100%)	0/0 (-)	7/7 (100%)	0/0 (-)	7/7 (100%)
Escherichia coli	10	1/1 (100%)	9/9 (100%)	0/0 (-)	10/10 (100%)	0/0 (-)	10/10 (100%)	0/0 (-)	10/10 (100%)	0/0 (-)	10/10 (100%)	1/1 (100%)	9/9 (100%)
Klebsiella aerogenes	3	0/0 (-)	3/3 (100%)	0/0 (-)	3/3 (100%)	0/0 (-)	3/3 (100%)	0/0 (-)	3/3 (100%)	0/0 (-)	3/3 (100%)	0/0 (-)	3/3 (100%)
Klebsiella oxytoca	4	0/0 (-)	4/4 (100%)	0/0 (-)	4/4 (100%)	0/0 (-)	4/4 (100%)	0/0 (-)	4/4 (100%)	0/0 (-)	4/4 (100%)	0/0 (-)	4/4 (100%)
Klebsiella pneumoniae group	21	1/1 (100%)	20/20 (100%)	0/0 (-)	21/21 (100%)	1/1 (100%)	20/20 (100%)	0/0 (-)	21/21 (100%)	0/0 (-)	21/21 (100%)	2/2 (100%)	19/19 (100%)
Proteus spp.	6	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)
Pseudomonas aeruginosa	68	0/1 (0%)	65/67 (97.0%)	0/0 (-)	68/68 (100%)	0/1 (0%)	67/67 (100%)	0/0 (-)	68/68 (100%)	0/0 (-)	68/68 (100%)	0/2 (0%)	64/66 (97.0%)
Serratia marcescens	14[e]	0/0 (-)	14/14 (100%)	1/1 (100%)	13/13 (100%)	0/0 (-)	14/14 (100%)	0/0 (-)	14/14 (100%)	0/0 (-)	14/14 (100%)	1/1 (100%)	13/13 (100%)
Polymicrobial specimens	92	1/1[f] (100%)	88/91[g] (96.7%)	0/0 (-)	92/92 (100%)	4/4[h] (100%)	87/88[i] (98.9%)	0/0 (-)	92/92 (100%)	1/1[j] (100%)	91/91 (100%)	5/5 (100%)	84/87 (96.6%)

[a] An additional 19 specimens had no applicable bacteria detected by BIOFIRE, but had one or more applicable bacteria isolated by qRefCx; CTX-M was identified by PCR/seq in one specimen (*E. coli* isolated by qRefCx), but no other resistance markers were identified in the cultured isolate(s) by PCR/seq from these specimens.

[b] One specimen had presence of dual AMR genes (KPC and VIM).

[c] An *A. calcoaceticus-baumannii* isolate was not recovered by qRefCx for one specimen.

[d] An *E. cloacae* isolate was not recovered by qRefCx for one specimen.

[e] A *S. marcescens* isolate was not recovered by qRefCx for one specimen.

[f] *E. coli* and *P. aeruginosa* detected by BIOFIRE and isolated by qRefCx (CTX-M identified in the *E. coli* isolate by PCR/seq).

[g] One specimen *A. calcoaceticus-baumannii* complex, *K. pneumoniae* group, *Proteus* spp., and *P. aeruginosa* detected by BIOFIRE (*K. pneumoniae* group and *P. aeruginosa* isolated by qRefCx; CTX-M was not identified in either of these isolates by PCR/seq); one specimen *E. coli*, *K. pneumoniae* group, and *P. aeruginosa* detected by BIOFIRE (*E. coli* and *P. aeruginosa* isolated by qRefCx; CTX-M was not identified in either of these isolated by PCR/seq); one specimen *Proteus* spp. and *P. aeruginosa* detected by BIOFIRE (*P. aeruginosa* isolated by qRefCx; CTX-M was not identified in this isolate by PCR/seq).

[h] One specimen *E. coli* and *K. pneumoniae* group detected by BIOFIRE and isolated by qRefCx (KPC identified in the *K. pneumoniae* isolate by PCR/seq); one specimen *P. aeruginosa* and *S. marcescens* detected by BIOFIRE and isolated by qRefCx (KPC identified in the *S. marcescens* isolate by PCR/seq); one specimen *A. calcoaceticus-baumannii* complex, *K. aerogenes*, *K. pneumoniae* group, and *P. aeruginosa* detected by BIOFIRE (*A. calcoaceticus-baumannii*, *K. pneumoniae*, and *P. aeruginosa* isolated by qRefCx; KPC identified in the *K. pneumoniae* isolate by PCR/seq); one specimen *A. calcoaceticus-baumannii* complex, *K. pneumoniae* group, *Proteus* spp., and *P. aeruginosa* detected by BIOFIRE (*K. pneumoniae* and *P. aeruginosa* isolated by qRefCx; KPC identified in the *K. pneumoniae* isolate by PCR/seq).

[i] One specimen *K. pneumoniae* group and *P. aeruginosa* detected by BIOFIRE (*P. aeruginosa* isolated by qRefCx; KPC was not identified in this isolate by PCR/seq).

[j] One specimen *A. calcoaceticus-baumannii* complex, *K. aerogenes*, *K. pneumoniae* group, and *P. aeruginosa* detected by BIOFIRE (*A. calcoaceticus-baumannii*, *K. pneumoniae*, and *P. aeruginosa* isolated by qRefCx; VIM identified in the *P. aeruginosa* isolate by PCR/seq).

qRefCx isolated one or more applicable gram-negative bacteria from 79 of the 94 BAL specimens and 131 of the 196 sputum specimens that received a BIOFIRE Pneumonia Panel Detected result for an applicable gram-negative bacterium for OXA-48-like. The method used to assess correlation of the OXA-48-like results (Table 27 and Table 28) reported in the specimen by the BIOFIRE Pneumonia Panel to identification of the gene in the cultured isolates from that particular specimen was one conventional PCR assay followed by bidirectional sequencing, performed directly on the isolate.

Table 27. OXA-48-like Performance Table (PCR/seq on cultured isolate(s) from BAL specimens)

BAL						
Applicable Bacteria Result	**Positive Percent Agreement**			**Negative Percent Agreement**		
	TP/(TP + FN)	**%**	**95%CI**	**TN/(TN + FP)**	**%**	**95%CI**
Overall *(any applicable bacteria Detected)*	***0/0***	***-***	***-***	***79/79***	***100***	***95.4-100%***
Enterobacter cloacae complex	0/0	-	-	10/10	100	72.2-100%
Escherichia coli	0/0	-	-	13/13	100	77.2-100%
Klebsiella aerogenes	0/0	-	-	7/7	100	64.6-100%
Klebsiella oxytoca	0/0	-	-	3/3	100	43.9-100%
Klebsiella pneumoniae group	0/0	-	-	15/15	100	79.6-100%
Proteus spp.	0/0	-	-	6/6	100	61.0-100%
Serratia marcescens	0/0	-	-	8/8	100	67.6-100%
Polymicrobial specimens	0/0	-	-	17/17	100	81.6-100%

Table 28. OXA-48-like Performance Table (PCR/seq on cultured isolate(s) from sputum specimens)

Sputum						
Applicable Bacteria Result	**Positive Percent Agreement**			**Negative Percent Agreement**		
	TP/(TP + FN)	**%**	**95%CI**	**TN/(TN + FP)**	**%**	**95%CI**
Overall *(any applicable bacteria Detected)*	***0/0***	***-***	***-***	***131/131***	***100***	***97.2-100%***
Enterobacter cloacae complex	0/0	-	-	9/9	100	70.1-100%
Escherichia coli	0/0	-	-	17/17	100	81.6-100%
Klebsiella aerogenes	0/0	-	-	4/4	100	51.0-100%
Klebsiella oxytoca	0/0	-	-	5/5	100	56.6-100%
Klebsiella pneumoniae group	0/0	-	-	25/25	100	86.7-100%
Proteus spp.	0/0	-	-	9/9	100	70.1-100%
Serratia marcescens	0/0	-	-	25/25	100	86.7-100%
Polymicrobial specimens	0/0	-	-	37/37	100	90.6-100%

qRefCx isolated *S. aureus* from 75 of 116 BAL specimens and 154 of 204 sputum specimens that received a BIOFIRE Pneumonia Panel *Staphylococcus aureus* Detected result. The method used to assess correlation of the *mecA/C* and MREJ (MRSA) results (Table 29 and Table 30) reported in the specimen by the BIOFIRE Pneumonia Panel to identification of the gene in the cultured isolates from that particular specimen was one conventional PCR assay followed by bidirectional sequencing, performed directly on the isolate.

Table 29. *mecA/C* and MREJ (MRSA)3x3 Performance Table (qRefCx & PCR/seq on cultured isolate(s) from BAL specimens)

BAL					
***S. aureus* *mecA/C* and MREJ (MRSA)**		**qRefCx: *S. aureus* PCR/seq: *mecA/C***			
		Org+ / Res+	**Org+ / Res-**	**Org -**	**Total**
FILMARRAY Result	**Org+ / Res+**	19	2	25	46
	Org+ / Res-	1	24	45	70
	Org -	0	1	729	730
	Total	20	27	799	846
Performance		**Agreement**	**%**	**95%CI**	
Org+ / Res+		19/20	95.0%	76.4-99.1%	
Org+ / Res-		24/27	88.9%	71.9-96.1%	
Org -		729/799	91.2%	89.1-93.0%	
Interpretation		**PPA**	**NPA**	**Prevalence**	
MRSA		19/20	799/826	46/846	
		(95.0%)	(96.7%)	(5.4%)	
MSSA		24/27	773/819	70/846	
		(88.9%)	(94.4%)	(8.3%)	
S. aureus		46/47	729/799	116/846	
		(97.9%)	(91.2%)	(13.7%)	

Table 30. *mecA/C* and MREJ (MRSA) 3x3 Performance Table (qRefCx & PCR/seq on cultured isolate(s) from sputum specimens)

Sputum					
***S. aureus* *mecA/C* and MREJ (MRSA)**		**qRefCx: *S. aureus* PCR/seq: *mecA/C***			
		Org+ / Res+	**Org+ / Res-**	**Org -**	**Total**
FILMARRAY Result	**Org+ / Res+**	58	4	45	107
	Org+ / Res-	0	49	48	97
	Org -	0	1	631	632
	Total	58	54	724	836
Performance		**Agreement**	**%**	**95%CI**	
Org+ / Res+		58/58	100%	93.8-100%	
Org+ / Res-		49/54	90.7%	80.1-96.0%	
Org -		631/724	87.2%	84.5-89.4%	
Interpretation		**PPA**	**NPA**	**Prevalence**	
MRSA		58/58	729/778	107/836	
		(100%)	(93.7%)	(12.8%)	
MSSA		49/54	734/782	97/836	
		(90.7%)	(93.9%)	(11.6%)	
S. aureus		111/112	631/724	204/836	
		(99.1%)	(87.2%)	(24.4%)	

BIOFIRE Pneumonia Panel CTX-M reporting was also compared to the standard phenotypic extended spectrum β-lactamase (ESBL) activity testing methods performed in conjunction with qRefCx. Standard phenotypic ESBL activity was only reported for *E. coli* and *Klebsiella* spp. by the central reference laboratory.

Of the 156 BAL specimens that received a BIOFIRE Pneumonia Panel Detected result for at least one applicable gram-negative bacterium on the panel, 53 specimens received a Detected result for *E. coli*, *K. oxytoca*, and/or *K. pneumoniae*; qRefCx isolated *E. coli*, *K. oxytoca*, and/or *K. pneumoniae* from 43 of these specimens. Of the 295 sputum specimens that received a BIOFIRE Pneumonia Panel Detected result for at least one applicable gram-negative bacterium on the panel, 114 specimens received a Detected result for *E. coli*, *K. oxytoca*, and/or *K. pneumoniae*; qRefCx isolated *E. coli*, *K. oxytoca*, and/or *K. pneumoniae* from 71 of these specimens. The correlation between BIOFIRE Pneumonia Panel reporting of CTX-M in a particular specimen as compared to the phenotypic AST results of isolates recovered from the same specimen are stratified by each applicable associated organism in Table 31 and Table 32.

Table 31. CTX-M Performance Table (comparison to phenotypic AST methods for BAL specimens)

BAL						
Applicable Bacteria Result	**Positive Percent Agreement**			**Negative Percent Agreement**		
	TP/(TP + FN)	**%**	**95%CI**	**TN/(TN + FP)**	**%**	**95%CI**
Overall *(any applicable bacteria Detected)*	***4/5***	***80.0***	***37.6-96.4%***	***38/38***	***100***	***90.8-100%***
Escherichia coli	4/4	100	51.0-100%	11/11	100	74.1-100%
Klebsiella oxytoca	0/0	-	-	5/5	100	56.6-100%
Klebsiella pneumoniae group	0/1	0	-	17/17	100	81.6-100%
Polymicrobial specimens	0/0	-	-	5/5	100	56.6-100%

Table 32. CTX-M Performance Table (comparison to phenotypic AST methods for sputum specimens)

Sputum						
Applicable Bacteria Result	**Positive Percent Agreement**			**Negative Percent Agreement**		
	TP/(TP + FN)	**%**	**95%CI**	**TN/(TN + FP)**	**%**	**95%CI**
Overall *(any applicable bacteria Detected)*	***4/7***	***57.1***	***25.1-84.2%***	***63/64***	***98.4***	***91.7-99.7%***
Escherichia coli	2/3	66.7	20.8-93.9%	17/17	100	81.6-100%
Klebsiella oxytoca	0/0	-	-	9/9	100	70.1-100%
Klebsiella pneumoniae group	1/2	50.0	-	26/27	96.3	81.7-99.3%
Polymicrobial specimens	1/2[a]	50.0	-	11/11	100	74.1-100%

[a] One specimen *E. coli* and *K. oxytoca* detected by BIOFIRE and isolated by qRefCx (ESBL activity identified in the *E. coli* isolate by qRefCx AST); one specimen *E. coli* and *K. pneumoniae* group detected by BIOFIRE (*E. coli* isolated by qRefCx and ESBL activity identified by qRefCx AST).

The BIOFIRE Pneumonia Panel carbapenem resistance gene reporting was also compared to the standard phenotypic carbapenem susceptibility testing methods performed in conjunction with qRefCx. In accordance with current CLSI guidelines, standard phenotypic ertapenem susceptibility is not reported for *A. calcoaceticus-baumannii* complex; therefore carbapenem susceptibility is only based on meropenem susceptibility for this organism. Resistance or intermediate-resistance to either ertapenem or meropenem constituted carbapenem resistance for this analysis. The correlation between BIOFIRE Pneumonia Panel reporting of the carbapenem resistance genes in a particular specimen as compared to the phenotypic AST results of isolates recovered from the same specimen are stratified by each applicable associated organism in Table 33 through Table 36.

Table 33. IMP, KPC, NDM, and VIM Performance Table (comparison to phenotypic AST methods for BAL specimens)

BAL											
Applicable Bacteria Result	**N**	**IMP**		**KPC**		**NDM**		**VIM**		***Overall*** (any carbapenem resistance gene)	
		PPA	NPA	PPA	NPA	PPA	NPA	PPA	NPA	PPA	NPA
Overall *(any applicable bacteria Detected)*	**126[a]**	**0/17 (0%)**	**109/109 (100%)**	**3/17 (17.6%)**	**109/109 (100%)**	**0/17 (0%)**	**109/109 (100%)**	**0/17 (0%)**	**109/109 (100%)**	**3/17 (17.6%) [6.2-41.0%]**	**109/109 (100%) [96.6-100%]**
Acinetobacter calcoaceticus-baumannii complex	0	-	-	-	-	-	-	-	-	-	-
Enterobacter cloacae complex	9	0/0 (-)	9/9 (100%)	0/0 (-)	9/9 (100%)	0/0 (-)	9/9 (100%)	0/0 (-)	9/9 (100%)	0/0 (-)	9/9 (100%)
Escherichia coli	12	0/0 (-)	12/12 (100%)	0/0 (-)	12/12 (100%)	0/0 (-)	12/12 (100%)	0/0 (-)	12/12 (100%)	0/0 (-)	12/12 (100%)
Klebsiella aerogenes	7	0/0 (-)	7/7 (100%)	0/0 (-)	7/7 (100%)	0/0 (-)	7/7 (100%)	0/0 (-)	7/7 (100%)	0/0 (-)	7/7 (100%)
Klebsiella oxytoca	2	0/0 (-)	2/2 (100%)	0/0 (-)	2/2 (100%)	0/0 (-)	2/2 (100%)	0/0 (-)	2/2 (100%)	0/0 (-)	2/2 (100%)
Klebsiella pneumoniae group	14	0/0 (-)	14/14 (100%)	0/0 (-)	14/14 (100%)	0/0 (-)	14/14 (100%)	0/0 (-)	14/14 (100%)	0/0 (-)	14/14 (100%)
Proteus spp.	6	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)
Pseudomonas aeruginosa	42	0/12 (0%)	30/30 (100%)	0/12 (0%)	30/30 (100%)	0/12 (0%)	30/30 (100%)	0/12 (0%)	30/30 (100%)	0/12 (0%)	30/30 (100%)
Serratia marcescens	6	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)
Polymicrobial specimens	28	0/5 (0%)	23/23 (100%)	3/5 (60.0%)	23/23 (100%)	0/5 (0%)	23/23 (100%)	0/5 (0%)	23/23 (100%)	3/5[b] (60.0%)	23/23 (100%)

[a] The isolate recovered from one specimen (*P. aeruginosa)* did not yield valid AST results on the VITEK® instrument.

[b] Of the three specimens that were concordant: one specimen *A. calcoaceticus-baumannii* complex and *K. pneumoniae* group detected by BIOFIRE (*A. calcoaceticus-baumannii* isolated by qRefCx, carbapenem resistance identified by qRefCx AST, and KPC not identified in the isolate by PCR/seq); one specimen *E. cloacae* complex and *P. aeruginosa* detected by BIOFIRE and isolated by qRefCx (carbapenem resistance identified in the *E. cloacae* isolate by qRefCx AST, and KPC identified in the *E. cloacae* isolate by PCR/seq); one specimen *Proteus* spp. and *P. aeruginosa* detected by BIOFIRE (*P. aeruginosa* isolated by qRefCx, carbapenem resistance identified by qRefCx AST, and KPC not identified in the isolate by PCR/seq). Of the two specimens that were not concordant: one specimen *A. calcoaceticus-baumannii* complex and *P. aeruginosa* detected by BIOFIRE (*P. aeruginosa* isolated by qRefCx, carbapenem resistance identified by qRefCx AST, and KPC not identified in the isolate by PCR/seq); one specimen *E. cloacae* complex and *K. aerogenes* detected by BIOFIRE (*E. cloacae* isolated by qRefCx, carbapenem resistance identified by qRefCx AST and KPC not identified in the isolate by PCR/seq).

Table 34. IMP, KPC, NDM, and VIM Performance Table (comparison to phenotypic AST methods for sputum specimens)

Sputum											
Applicable Bacteria Result	**N**	**IMP**		**KPC**		**NDM**		**VIM**		***Overall*** (any carbapenem resistance gene)	
		PPA	NPA	PPA	NPA	PPA	NPA	PPA	NPA	PPA	NPA
Overall *(any applicable bacteria Detected)*	**229[a]**	**0/35 (0%)**	**194/194 (100%)**	**6/35 (17.1%)**	**193/194 (99.5%)**	**0/35 (0%)**	**194/194 (100%)**	**1/35 (2.9%)**	**194/194 (100%)**	**6/35[b] (17.1%) [8.1-32.7%]**	**193/194 (99.5%) [97.1-99.9%]**
Acinetobacter calcoaceticus-baumannii complex	5[c]	0/2 (0%)	3/3 (100%)	0/2 (0%)	3/3 (100%)	0/2 (0%)	3/3 (100%)	0/2 (0%)	3/3 (100%)	0/2 (0%)	3/3 (100%)
Enterobacter cloacae complex	7[d]	0/1 (0%)	6/6 (100%)	0/1 (0%)	6/6 (100%)	0/1 (0%)	6/6 (100%)	0/1 (0%)	6/6 (100%)	0/1 (0%)	6/6 (100%)
Escherichia coli	10	0/0 (-)	10/10 (100%)	0/0 (-)	10/10 (100%)	0/0 (-)	10/10 (100%)	0/0 (-)	10/10 (100%)	0/0 (-)	10/10 (100%)
Klebsiella aerogenes	3	0/0 (-)	3/3 (100%)	0/0 (-)	3/3 (100%)	0/0 (-)	3/3 (100%)	0/0 (-)	3/3 (100%)	0/0 (-)	3/3 (100%)
Klebsiella oxytoca	4	0/0 (-)	4/4 (100%)	0/0 (-)	4/4 (100%)	0/0 (-)	4/4 (100%)	0/0 (-)	4/4 (100%)	0/0 (-)	4/4 (100%)
Klebsiella pneumoniae group	21	0/2 (0%)	19/19 (100%)	1/2 (50.0%)	19/19 (100%)	0/2 (0%)	19/19 (100%)	0/2 (0%)	19/19 (100%)	1/2 (50.0%)	19/19 (100%)
Proteus spp.	6	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)	0/0 (-)	6/6 (100%)
Pseudomonas aeruginosa	67	0/16 (0%)	51/51 (100%)	0/16 (0%)	51/51 (100%)	0/16 (0%)	51/51 (100%)	0/16 (0%)	51/51 (100%)	0/16 (0%)	51/51 (100%)
Serratia marcescens	14[e]	0/1 (0%)	13/13 (100%)	1/1 (100%)	13/13 (100%)	0/1 (0%)	13/13 (100%)	0/1 (0%)	13/13 (100%)	1/1 (100%)	13/13 (100%)
Polymicrobial specimens	92	0/13 (0%)	79/79 (100%)	4/13 (30.8%)	78/79 (98.7%)	0/13 (0%)	79/79 (100%)	1/13 (7.7%)	79/79 (100%)	4/13[f] (30.8%)	78/79 (98.7%)

[a] The isolate recovered from one specimen (*P. aeruginosa)* did not yield valid AST results on the VITEK® instrument.

[b] One specimen had presence of dual AMR genes (KPC and VIM).

[c] An *A. calcoaceticus-baumannii* isolate was not recovered by qRefCx for one specimen.

[d] An *E. cloacae* isolate was not recovered by qRefCx for one specimen.

[e] A *S. marcescens* isolate was not recovered by qRefCx for one specimen.

[f] Of the four specimens that were concordant: one specimen *A. calcoaceticus-baumannii* complex, *K. aerogenes*, *K. pneumoniae* group, and *P. aeruginosa* detected by BIOFIRE (*A. calcoaceticus-baumannii*, *K. pneumoniae*, and *P. aeruginosa* isolated by qRefCx, carbapenem resistance identified in all three isolates by qRefCx AST, KPC identified in the *K. pneumoniae* isolate by PCR/seq, and VIM identified in the *P. aeruginosa* isolate by PCR/seq); one specimen *A. calcoaceticus-baumannii* complex, *K. pneumoniae* group, *Proteus* spp., and *P. aeruginosa* detected by BIOFIRE (*K. pneumoniae* and *P. aeruginosa* isolated by qRefCx, carbapenem resistance identified in the *K. pneumoniae* isolate by qRefCx AST, and KPC identified in the *K. pneumoniae* isolate by PCR/seq); one specimen *E. coli* and *K. pneumoniae* group detected by BIOFIRE and isolated by qRefCx (carbapenem resistance identified in the *K. pneumoniae* isolate by qRefCx AST and KPC identified in the isolate by PCR/seq); one specimen *P. aeruginosa* and *S. marcescens* detected by BIOFIRE and isolated by qRefCx (carbapenem resistance identified in the *S. marcescens* isolate by qRefCx AST and KPC identified in the isolate by PCR/seq). Of the nine specimens that were not concordant: one specimen *A. calcoaceticus-baumannii* complex and *Proteus* spp. detected by BIOFIRE and isolated by qRefCx (carbapenem resistance identified in the *A. calcoaceticus-baumannii* isolate by qRefCx AST and KPC not identified in either isolate by PCR/seq); one specimen *E. cloacae* complex and *P. aeruginosa* detected by BIOFIRE (*P. aeruginosa* isolated by qRefCx, carbapenem resistance identified by qRefCx AST, and KPC not identified in the isolate by PCR/seq); one specimen *E. coli* and *P. aeruginosa* detected by BIOFIRE and isolated by qRefCx (carbapenem resistance identified in the *P. aeruginosa* isolate by qRefCx AST and KPC not identified in either isolate by PCR/seq); one specimen *K. pneumoniae* group and *P. aeruginosa* detected by BIOFIRE and isolated by qRefCx (carbapenem resistance identified in the *K. pneumoniae* isolate by qRefCx AST and KPC not identified in either isolate by PCR/seq); one specimen *Proteus* spp. and *P. aeruginosa* detected by BIOFIRE and isolated by qRefCx (carbapenem resistance identified in the *P. aeruginosa* isolate by qRefCx AST and KPC not identified in either isolate by PCR/seq); one specimen *P. aeruginosa* and *S. marcescens* detected by BIOFIRE and isolated by qRefCx (carbapenem resistance identified in the *S. marcescens* isolate by qRefCx AST and KPC not identified in either isolate by PCR/seq); one specimen *P. aeruginosa* and *S. marcescens* detected by BIOFIRE (*P. aeruginosa* isolated by qRefCx, carbapenem resistance identified by qRefCx AST, and KPC not identified in the isolate by PCR/seq); one specimen *A. calcoaceticus-baumannii* complex, *E. coli, K. pneumoniae* group, and *P. aeruginosa* detected by BIOFIRE (*E. coli* and *P. aeruginosa* isolated by qRefCx, carbapenem resistance identified in the *P. aeruginosa*

isolate by qRefCx AST, and KPC not identified in either isolate by PCR/seq); one specimen *A. calcoaceticus-baumannii* complex, *Proteus* spp., *P. aeruginosa*, and *S. marcescens* detected by BIOFIRE (*Proteus* spp. and *P. aeruginosa* isolated by qRefCx; carbapenem resistance identified in the *P. aeruginosa* isolate by qRefCx AST and KPC not identified in either isolate by PCR/seq).

Table 35. OXA-48-like Performance Table (comparison to phenotypic AST methods for BAL specimens)

BAL						
Applicable Bacteria Result	**Positive Percent Agreement**			**Negative Percent Agreement**		
	TP/(TP + FN)	**%**	**95%CI**	**TN/(TN + FP)**	**%**	**95%CI**
Overall *(any applicable bacteria Detected)*	***0/2***	***0***	***-***	***77/77***	***100***	***95.2-100%***
Enterobacter cloacae complex	0/1	0	-	9/9	100	70.1-100%
Escherichia coli	0/0	-	-	13/13	100	77.2-100%
Klebsiella aerogenes	0/0	-	-	7/7	100	64.6-100%
Klebsiella oxytoca	0/0	-	-	3/3	100	43.9-100%
Klebsiella pneumoniae group	0/0	-	-	15/15	100	79.6-100%
Proteus spp.	0/0	-	-	6/6	100	61.0-100%
Serratia marcescens	0/0	-	-	8/8	100	67.6-100%
Polymicrobial specimens	0/1[a]	0	-	16/16	100	80.6-100%

[a] *E. cloacae* complex and *K. aerogenes* detected by BIOFIRE (*E. cloacae* complex isolated by qRefCx and carbapenem resistance identified by qRefCx AST).

Table 36. OXA-48-like Performance Table (comparison to phenotypic AST methods for sputum specimens)

Sputum						
Applicable Bacteria Result	**Positive Percent Agreement**			**Negative Percent Agreement**		
	TP/(TP + FN)	**%**	**95%CI**	**TN/(TN + FP)**	**%**	**95%CI**
Overall *(any applicable bacteria Detected)*	***0/10***	***0***	***-***	***121/121***	***100***	***96.9-100%***
Enterobacter cloacae complex	0/1	0	-	8/8	100	67.6-100%
Escherichia coli	0/0	-	-	17/17	100	81.6-100%
Klebsiella aerogenes	0/0	-	-	4/4	100	51.0-100%
Klebsiella oxytoca	0/0	-	-	5/5	100	56.6-100%
Klebsiella pneumoniae group	0/3	0	-	22/22	100	85.1-100%
Proteus spp.	0/0	-	-	9/9	100	70.1-100%
Serratia marcescens	0/3	0	-	22/22	100	85.1-100%
Polymicrobial specimens	0/3[a]	0	-	34/34	100	89.8-100%

[a] One specimen *E. coli* and *K. pneumoniae* group detected by BIOFIRE and isolated by qRefCx (carbapenem resistance identified in the *K. pneumoniae* group isolate by qRefCx AST); one specimen *K. aerogenes* and *K. pneumoniae* group detected by BIOFIRE (*K. pneumoniae* group isolated by qRefCx and carbapenem resistance identified by qRefCx AST); one specimen *K. pneumoniae* group and *Proteus* spp. detected by BIOFIRE (*K. pneumoniae* group isolated by qRefCx and carbapenem resistance identified by qRefCx AST).

The BIOFIRE Pneumonia Panel *mecA/C* and MREJ (MRSA) reporting was also compared to the standard phenotypic cefoxitin susceptibility testing methods performed in conjunction with qRefCx. The correlation between BIOFIRE Pneumonia Panel reporting of *mecA/C* and MREJ (MRSA) in a particular specimen as compared to the phenotypic AST results of isolates recovered from the same specimen is shown in Table 37 and Table 38.

Table 37. *mecA/C* and MREJ (MRSA) 3x3 Performance Table (qRefCx & phenotypic AST on cultured isolate(s) from BAL specimens)

BAL					
***S. aureus* *mecA/C* and MREJ (MRSA)**		**qRefCx:** *S. aureus* **qRefCx Phenotypic AST:** cefoxitin susceptibility			
		Org+ / Res+	Org+ / Res-	Org -	Total
BIOFIRE Result	Org+ / Res+	18	3	25	46
	Org+ / Res-	1	24	45	70
	Org -	0	1	729	730
	Total	19	28	799	846
Performance			**Agreement**	**%**	**95%CI**
Org+ / Res+			18/19	94.7%	75.4-99.1%
Org+ / Res-			24/28	85.7%	68.5-94.3%
Org -			729/799	91.2%	89.1-93.0%
Interpretation			**PPA**	**NPA**	**Prevalence**
MRSA			18/19	799/827	46/846
			(94.7%)	(96.6%)	(5.4%)
MSSA			24/28	772/818	70/846
			(85.7%)	(94.4%)	(8.3%)
S. aureus			46/47	729/799	116/846
			(97.9%)	(91.2%)	(13.7%)

Table 38. *mecA/C* and MREJ (MRSA) 3x3 Performance Table (qRefCx & phenotypic AST on cultured isolate(s) from sputum specimens)

Sputum					
***S. aureus* *mecA/C* and MREJ (MRSA)**		**qRefCx:** *S. aureus* **qRefCx Phenotypic AST:** cefoxitin susceptibility			
		Org+ / Res+	Org+ / Res-	Org -	Total
BIOFIRE Result	Org+ / Res+	59	3	45	107
	Org+ / Res-	1	48	48	97
	Org -	0	1	631	632
	Total	60	52	724	836
Performance			**Agreement**	**%**	**95%CI**
Org+ / Res+			59/60	98.3%	91.1-99.7%
Org+ / Res-			48/52	92.3%	81.8-97.0%
Org -			631/724	87.2%	84.5-89.4%
Interpretation			**PPA**	**NPA**	**Prevalence**
MRSA			59/60	728/776	107/836
			(98.3%)	(93.8%)	(12.8%)
MSSA			48/52	735/784	97/836
			(92.3%)	(93.8%)	(11.6%)
S. aureus			111/112	631/724	204/836
			(99.1%)	(87.2%)	(24.4%)

The BIOFIRE Pneumonia Panel bin performance compared to the quantitative molecular assay (qMol) comparator is shown for BAL (Table 39) and sputum (Table 40). The qMol values are broken into one-log ranges correlating to the reported semi-quantitative BIOFIRE Pneumonia Panel bins. The relationship between qMol quantitative bins in copies/mL and traditional culture quantification in CFU/mL is unknown.

Table 39. BIOFIRE Pneumonia Panel Overall bin performance for BAL Specimens (qMol)

BAL							
qMol Binned Values[a] (copies/mL)		**ND to <10^3.5**	**10^4.0**	**10^5.0**	**10^6.0**	**≥10^7.0**	***Total***
BIOFIRE Bin (copies/mL)	ND	12025	35	5	0	2	***12067***
	10^4	47	48	21	0	1	***117***
	10^5	5	23	57	22	2	***109***
	10^6	3	3	29	40	13	***88***
	≥10^7	2	0	4	41	112	***159***
% concordant		**12025/12082 (99.5%)**	**48/109 (44.0%)**	**57/116 (49.1%)**	**40/103 (38.8%)**	**112/130 (86.2%)**	***12540***
			257/458 (56.1%)				

[a] Shaded cells indicate results considered concordant between the BIOFIRE Pneumonia Panel and qMol.

Table 40. BIOFIRE Pneumonia Panel Overall bin performance for Sputum Specimens (qMol)

Sputum							
qMol Range of Values [a] (copies/mL)		**ND to <10^3.5**	**10^4.0**	**10^5.0**	**10^6.0**	**≥10^7.0**	***Total***
BIOFIRE Bin (copies/mL)	ND	11392	85	17	2	2	***11498***
	10^4	79	87	41	7	0	***214***
	10^5	12	33	104	43	5	***197***
	10^6	2	4	39	88	41	***174***
	≥10^7	4	0	1	44	288	***337***
% concordant		**11392/11489 (99.2%)**	**87/209 (41.6%)**	**104/202 (51.5%)**	**88/184 (47.8%)**	**288/336 (67.9%)**	***12420***
			567/931 (60.9%)				

[a] Shaded cells indicate results considered concordant between the BIOFIRE Pneumonia Panel and qMol.

The BIOFIRE Pneumonia Panel bin performance compared to qRefCx quantification is shown for BAL and sputum in Table 41 - Table 52. Data is shown for overall performance, as well as for some individual organisms. In these tables, the values reported by culture are broken into ranges. A BIOFIRE Pneumonia Panel bin result is considered concordant if the culture value is within 0.5 log of the bin boundary. For example, the 10^5 BIOFIRE Pneumonia Panel bin (10^4.5-10^5.5) is concordant with the culture range of 10^4-10^6 CFU/mL.

Table 41. BIOFIRE Pneumonia Panel Overall bin performance for BAL Specimens (qRefCx)

BAL							
qRefCx Range of Values [CFU/mL] (Predicted BIOFIRE Bin)		**ND to <10^3.5** (ND)	**10^3.5 to <10^4.0** (10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)
BIOFIRE Bin (copies/mL)	ND	12202	1	2	0	0	0
	10^4	116	1	3	0	0	0
	10^5	90	10	11	0	1	0
	10^6	61	10	17	2	1	0
	≥10^7	61	10	36	32	11	12

% concordant	**12202/12530 (97.4%)**	**1/32 (3.1%)**	**14/69 (20.3%)**	**2/34 (5.9%)**	**12/13 (92.3%)**	**12/12 (100%)**
		41/160 (25.6%)				

Table 42. BIOFIRE Pneumonia Panel Overall bin performance for Sputum Specimens (qRefCx)

Sputum							
qRefCx Range of Values [CFU/mL] (Predicted BIOFIRE Bin)		**ND to <10^3.5** (ND)	**10^3.5 to <10^4.0** (10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)
BIOFIRE Bin (copies/mL)	ND	11596	4	6	2	0	1
	10^4	193	14	9	0	0	0
	10^5	139	19	28	14	1	0
	10^6	94	19	36	21	4	1
	≥10^7	121	8	88	53	49	20
% concordant		**11596/12143 (95.5%)**	**14/64 (21.9%)**	**37/167 (22.2%)**	**35/90 (38.9%)**	**53/54 (98.1%)**	**20/22 (90.9%)**
			159/397 (40.1%)				

Table 43. BIOFIRE Pneumonia Panel *H. influenzae* bin performance for BAL Specimens (qRefCx)

BAL							
qRefCx Range of Values [CFU/mL] (Predicted BIOFIRE Bin)		**ND to <10^3.5** (ND)	**10^3.5 to <10^4.0** (10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)
BIOFIRE Bin (copies/mL)	ND	764	0	0	0	0	0
	10^4	17	0	0	0	0	0
	10^5	12	0	0	0	0	0
	10^6	13	2	0	0	0	0
	≥10^7	30	0	2	5	0	1
% concordant		**764/836 (91.4%)**	**0/2 (0%)**	**0/2 (0%)**	**0/5 (0%)**	**0/0 (-)**	**1/1 (100%)**
			1/10 (10.0%)				

Table 44. BIOFIRE Pneumonia Panel *H. influenzae* bin performance for Sputum Specimens (qRefCx)

Sputum							
qRefCx Range of Values [CFU/mL] (Predicted BIOFIRE Bin)		**ND to <10^3.5** (ND)	**10^3.5 to <10^4.0** (10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)
BIOFIRE Bin (copies/mL)	ND	727	0	1	1	0	0
	10^4	21	0	0	0	0	0
	10^5	19	0	0	1	0	0
	10^6	13	0	1	0	0	0
	≥10^7	38	0	10	2	2	0
% concordant		**727/818 (88.9%)**	**0/0 (-)**	**0/12 (0%)**	**1/4 (25.0%)**	**2/2 (100%)**	**0/0 (-)**
			3/18 (16.7%)				

Table 45. BIOFIRE Pneumonia Panel *P. aeruginosa* bin performance for BAL Specimens (qRefCx)

BAL							
qRefCx Range of Values [CFU/mL] (Predicted BIOFIRE Bin)		**ND to <10^3.5** (ND)	**10^3.5 to <10^4.0** (10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)
BIOFIRE Bin (copies/mL)	ND	772	0	0	0	0	0
	10^4	12	0	1	0	0	0
	10^5	14	2	1	0	0	0
	10^6	5	1	2	1	1	0
	≥10^7	7	1	11	12	1	2
% concordant		**772/810 (95.3%)**	**0/4 (0.0%)**	**2/15 (13.3%)**	**1/13 (7.7%)**	**2/2 (100%)**	**2/2 (100%)**
			7/36 (19.4%)				

Table 46. BIOFIRE Pneumonia Panel *P. aeruginosa* bin performance for Sputum Specimens (qRefCx)

Sputum							
qRefCx Range of Values [CFU/mL] (Predicted BIOFIRE Bin)		**ND to <10^3.5** (ND)	**10^3.5 to <10^4.0** (10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)
BIOFIRE Bin (copies/mL)	ND	673	2	1	0	0	0
	10^4	16	3	1	0	0	0
	10^5	17	3	6	2	0	0
	10^6	12	4	8	3	1	0
	≥10^7	12	3	26	19	18	6
% concordant		**673/730 (92.2%)**	**3/15 (20.0%)**	**7/42 (16.7%)**	**5/24 (20.8%)**	**19/19 (100%)**	**6/6 (100%)**
			40/106 (37.7%)				

Table 47. BIOFIRE Pneumonia Panel *S. aureus* bin performance for BAL Specimens (qRefCx)

BAL							
qRefCx Range of Values [CFU/mL] (Predicted BIOFIRE Bin)		**ND to <10^3.5** (ND)	**10^3.5 to <10^4.0** (10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)
BIOFIRE Bin (copies/mL)	ND	729	1	0	0	0	0
	10^4	33	0	0	0	0	0
	10^5	23	3	0	0	0	0
	10^6	13	2	7	1	0	0
	≥10^7	1	5	12	8	5	3
% concordant		**729/799 (91.2%)**	**0/11 (0.0%)**	**0/19 (0.0%)**	**1/9 (11.1%)**	**5/5 (100%)**	**3/3 (100%)**
			9/47 (19.1%)				

Table 48. BIOFIRE Pneumonia Panel *S. aureus* bin performance for Sputum Specimens (qRefCx)

Sputum							
qRefCx Range of Values [CFU/mL] (Predicted BIOFIRE Bin)		**ND to <10^3.5** (ND)	**10^3.5 to <10^4.0** (10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)
BIOFIRE Bin (copies/mL)	ND	631	1	0	0	0	0
	10^4	39	1	3	0	0	0
	10^5	33	7	8	4	1	0

Sputum							
qRefCx Range of Values [CFU/mL] (Predicted BIOFIRE Bin)		**ND to <10^3.5** (ND)	**10^3.5 to <10^4.0** (10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)
	10^6	12	7	13	9	1	1
	≥10^7	9	2	21	15	11	7
% concordant		**631/724 (87.2%)**	**1/18 (5.6%)**	**11/45 (24.4%)**	**13/28 (46.4%)**	**12/13 (92.3%)**	**7/8 (87.5%)**
			44/112 (39.3%)				

Table 49. BIOFIRE Pneumonia Panel *S. pneumoniae* bin performance for BAL Specimens (qRefCx)

BAL							
qRefCx Range of Values [CFU/mL] (Predicted BIOFIRE Bin)		**ND to <10^3.5** (ND)	**10^3.5 to <10^4.0** (10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)
BIOFIRE Bin (copies/mL)	ND	817	0	0	0	0	0
	10^4	8	1	0	0	0	0
	10^5	7	0	1	0	0	0
	10^6	5	0	1	0	0	0
	≥10^7	4	1	0	1	0	0
% concordant		**817/841 (97.1%)**	**1/2 (50.0%)**	**1/2 (50.0%)**	**0/1 (0.0%)**	**0/0 (-%)**	**0/0 (-%)**
			2/5 (40%)				

Table 50. BIOFIRE Pneumonia Panel *S. pneumoniae* bin performance for Sputum Specimens (qRefCx)

Sputum							
qRefCx Range of Values [CFU/mL] (Predicted BIOFIRE Bin)		**ND to <10^3.5** (ND)	**10^3.5 to <10^4.0** (10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)
BIOFIRE Bin (copies/mL)	ND	785	0	0	0	0	0
	10^4	10	2	0	0	0	0
	10^5	8	0	2	0	0	0
	10^6	9	1	0	0	0	0
	≥10^7	8	0	4	2	4	1
% concordant		**785/820 (95.7%)**	**2/3 (66.7%)**	**2/6 (33.3%)**	**0/2 (0.0%)**	**4/4 (100%)**	**1/1 (100%)**
			9/16 (56.3%)				

Table 51. BIOFIRE Pneumonia Panel Bin Performance Summary for BAL Specimens comparted to qRefCx

BAL						
qRefCx Range of Values (Concordant BIOFIRE Bin)	**10^3.5 to <10^4.0** (10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)	**Overall**
***Acinetobacter calcoaceticus-baumannii* complex**	0/0 (-)	0/0 (-)	0/0 (-)	0/0 (-)	0/0 (-)	0/0 (-)
***Enterobacter cloacae* complex**	0/1 (0%)	1/7 (14.3%)	0/3 (0%)	0/0 (-)	1/1 (100%)	2/12 (16.7%)
Escherichia coli	0/4 (0%)	1/7 (14.3%)	0/0 (-)	0/0 (-)	1/1 (100%)	2/12 (16.7%)
Haemophilus influenzae	0/2 (0%)	0/2 (0%)	0/5 (0%)	0/0 (-)	1/1 (100%)	1/10 (10.0%)
Klebsiella aerogenes	0/1 (0%)	1/2 (50.0%)	0/1 (0%)	2/3 (66.7%)	0/0 (-)	3/7 (42.9%)

BAL						
qRefCx Range of Values (Concordant BIOFIRE Bin)	**10^3.5 to <10^4.0** (10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)	**Overall**
Klebsiella oxytoca	0/0 (-)	1/2 (50.0%)	0/0 (-)	0/0 (-)	0/0 (-)	1/2 (50.0%)
***Klebsiella pneumoniae* group**	0/5 (0%)	3/5 (60.0%)	0/0 (-)	2/2 (100%)	3/3 (100%)	8/15 (53.3%)
Moraxella catarrhalis	0/0 (-)	0/0 (-)	0/0 (-)	0/0 (-)	0/0 (-)	0/0 (-)
***Proteus* spp.**	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	3/5 (60.0%)
Pseudomonas aeruginosa	0/4 (0%)	2/15 (13.3%)	1/13 (7.7%)	2/2 (100%)	2/2 (100%)	7/36 (19.4%)
Serratia marcescens	0/1 (0%)	1/4 (25.0%)	0/1 (0%)	0/0 (-)	0/0 (-)	1/6 (16.7%)
Staphylococcus aureus	0/11 (0.0%)	0/19 (0%)	1/9 (11.1%)	5/5 (100%)	3/3 (100%)	9/47 (19.1%)
Streptococcus agalactiae	0/0 (-)	0/1 (0%)	0/0 (-)	0/0 (-)	0/0 (-)	0/1 (0%)
Streptococcus pneumoniae	1/2 (50.0%)	1/2 (50.0%)	0/1 (0%)	0/0 (-)	0/0 (-)	2/5 (40.0%)
Streptococcus pyogenes	0/0 (-)	2/2 (100%)	0/0 (-)	0/0 (-)	0/0 (-)	2/2 (100%)

Table 52. BIOFIRE Pneumonia Panel Bin Performance Summary for Sputum Specimens comparted to qRefCx

Sputum						
qRefCx Range of Values (Concordant BIOFIRE Bin)	**10^3.5 to <10^4.0** (10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)	**Overall**
***Acinetobacter calcoaceticus-baumannii* complex**	1/5 (20.0%)	1/1 (100%)	0/2 (0%)	3/3 (100%)	0/0 (-)	5/11 (45.5%)
***Enterobacter cloacae* complex**	0/1 (0%)	3/6 (50.0%)	1/3 (33.3%)	1/1 (100%)	1/1 (100%)	6/12 (50.0%)
Escherichia coli	2/3 (66.7%)	1/13 (7.7%)	2/5 (40.0%)	1/1 (100%)	2/2 (100%)	8/24 (33.3%)
Haemophilus influenzae	0/0 (-)	0/12 (0%)	1/4 (25.0%)	2/2 (100%)	0/0 (-)	3/18 (16.7%)
Klebsiella aerogenes	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/0 (-)	2/4 (50.0%)
Klebsiella oxytoca	1/3 (33.3%)	0/3 (0%)	2/2 (100%)	1/1 (100%)	0/0 (-)	4/9 (44.4%)
***Klebsiella pneumoniae* group**	3/6 (50.0%)	2/7 (28.6%)	5/7 (71.4%)	2/2 (100%)	0/1 (0%)	12/23 (52.2%)
Moraxella catarrhalis	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	2/5 (40.0%)
***Proteus* spp.**	0/1 (0%)	5/10 (50.0%)	2/3 (66.7%)	1/1 (100%)	0/0 (-)	8/15 (53.3%)
Pseudomonas aeruginosa	3/15 (20.0%)	7/42 (16.7%)	5/24 (20.8%)	19/19 (100%)	6/6 (100%)	40/106 (37.7%)
Serratia marcescens	0/4 (0%)	4/12 (33.3%)	3/6 (50.0%)	4/4 (100%)	1/1 (100%)	12/27 (44.4%)
Staphylococcus aureus	1/18 (5.6%)	11/45 (24.4%)	13/28 (46.4%)	12/13 (92.3%)	7/8 (87.5%)	44/112 (39.3%)
Streptococcus agalactiae	0/3 (0%)	1/5 (20.0%)	0/1 (0%)	0/0 (-)	0/0 (-)	1/9 (11.1%)
Streptococcus pneumoniae	2/3 (66.7%)	2/6 (33.3%)	0/2 (0%)	4/4 (100%)	1/1 (100%)	9/16 (56.3%)
Streptococcus pyogenes	0/0	0/3	1/1	1/1	1/1	3/6

Sputum						
qRefCx Range of Values (Concordant BIOFIRE Bin)	**10^3.5 to <10^4.0** (10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)	**Overall**
	(-)	(0%)	(100%)	(100%)	(100%)	(50.0%)

A 'rank order' analysis was performed on polymicrobial specimens to compare the relative abundance of each analyte within a specimen as reported by qRefCx to the order reported by the BIOFIRE Pneumonia Panel (i.e. relative level of each analyte in a polymicrobial specimen, ranked from most to least abundant). In the prospective study, there were 20 BAL and 84 sputum specimens with two or more organisms reported by qRefCx. In these specimens, the BIOFIRE Pneumonia Panel was in agreement with qRefCx for the most abundant organism 45.0% of the time (9/20) for BAL and 41.7% of the time (35/84) for sputum. For the second most abundant organism, the BIOFIRE Pneumonia Panel was in agreement with qRefCx 30.0% of the time (6/20) for BAL and 26.2% of the time (22/84) for sputum, and was in agreement for the third most abundant organism 7.7% of the time (1/13) for BAL and 3.8% of the time (2/52) for sputum. False positives results were always considered discordant (i.e. only exact rank matches are considered concordant).

Table 53. Concordance of Abundance in Polymicrobial Specimens (as compared to qRefCx)

Ranking Performance	BAL			Sputum		
	Correct	Total	%	Correct	Total	%
Most Abundant	9	20	45.0%	35	84	41.7%
Second Most Abundant	6	20	30.0%	22	84	26.2%
Third Most Abundant	1	13	7.7%	2	52	3.8%

'Rank concordance' analysis was performed on polymicrobial specimens to determine the ability of the BIOFIRE Pneumonia Panel to measure relative abundance of the nucleic acid for an organism with respect to other organisms in the specimen, as compared to qRefCx. In this analysis, the detected organisms from individual polymicrobial specimens (110 BAL and 246 sputum) were ranked in descending order based on their quantification values from qRefCx. The rank determined by the BIOFIRE Pneumonia Panel bin result was compared to the qRefCx ranking. False positives results were always considered discordant (i.e. only exact rank matches are considered concordant).

Table 54. Concordance of Organism Ranking in Polymicrobial Specimens (as compared to qRefCx); false positive results considered discordant

Ranking Performance	BAL			Sputum		
	Concordant	Total	%	Concordant	Total	%
***Acinetobacter calcoaceticus-baumannii* complex**	1	6	16.7%	7	25	28.0%
***Enterobacter cloacae* complex**	9	14	64.3%	9	25	36.0%
Escherichia coli	6	13	46.2%	14	39	35.9%
Haemophilus influenzae	9	47	19.1%	10	62	16.1%
Klebsiella aerogenes	4	9	44.4%	4	9	44.4%
Klebsiella oxytoca	3	10	30.0%	5	17	29.4%
***Klebsiella pneumoniae* group**	8	16	50.0%	15	43	34.9%
Moraxella catarrhalis	0	15	0.0%	3	59	5.1%
***Proteus* spp.**	2	6	33.3%	5	20	25.0%
Pseudomonas aeruginosa	17	25	68.0%	59	107	55.1%
Serratia marcescens	5	10	50.0%	17	43	39.5%
Staphylococcus aureus	30	55	54.5%	59	141	41.8%
Streptococcus agalactiae	6	19	31.6%	10	35	28.6%
Streptococcus pneumoniae	4	23	17.4%	7	37	18.9%

Streptococcus pyogenes	1	5	20.0%	2	5	40.0%

The overall success rate for initial specimen tests in the prospective study was 98.1% (1764/1798); 34 tests were unsuccessful (two due to an incomplete test and 32 due to control failures). Two tests (2/1798; 0.1%) did not complete on the initial run, resulting in an instrument success rate of 99.9% (1796/1798) for initial specimen tests. Both specimens were able to be retested and valid results were produced after a single retest.

Thirty-two (32) tests (32/1764; 1.8%) did not produce valid pouch controls, resulting in a pouch control success rate of 98.2% (1764/1796) for completed runs in the initial specimen tests. Twenty-eight (28) of the 32 invalid specimens were able to be retested; 25 produced valid control results after a single retest, while the remaining three did not produce valid control results after retesting and were not able to be retested further due to insufficient specimen volume; four were not able to be retested at all due to insufficient specimen volume.

Two additional studies were also conducted to demonstrate all aspects of clinical performance (see Testing of Preselected Archived Specimens and Testing of Contrived Specimens below).

Testing of Preselected Archived Specimens

Some of the analytes on the BIOFIRE Pneumonia Panel were of low prevalence and were not encountered in large enough numbers during the prospective study to adequately demonstrate system performance. To supplement the results of the prospective clinical study, an evaluation of preselected archived retrospective specimens was performed at BIOFIRE.

A total of 171 frozen archived specimens were received for testing from external laboratories. Eighteen (18) specimens were negatives (13 BAL and 5 sputum) and 153 specimens (139 BAL and 14 sputum) contained at least one analyte of interest. Twenty-two (22) specimens contained two or more analytes of interest. The set included specimens known to be positive for: *Acinetobacter calcoaceticus-baumannii* complex, *Klebsiella aerogenes*, *Klebsiella oxytoca*, *Proteus* spp., *Serratia marcescens*, *Streptococcus pyogenes*, *Chlamydia pneumoniae*, *Legionella pneumophila*, adenovirus, human metapneumovirus, influenza A virus, influenza B virus, parainfluenza virus (PIV), respiratory syncytial virus, various gram-negative bacteria with extended-spectrum β-lactamase (ESBL) phenotype, and various gram-negative bacteria with a carbapenem resistant phenotype.

Prior to testing with the BIOFIRE Pneumonia Panel, the composition/integrity of the specimens was first confirmed with confirmatory molecular methods. At the completion of testing, four specimens were excluded due to invalid confirmation tests; these specimens had insufficient volume for retesting. Results from the remaining 18 negative and 149 positive specimens (containing 173 analytes) are presented here.

The reported analyte (as determined by the source laboratory) was confirmed for 117 analytes (107 in BAL and 10 in sputum) of the expected positive results (117/173; 67.6 %). More than three quarters of the unconfirmed analytes were specimens previously identified as positive for gram-negative bacteria exhibiting phenotypic ESBL or carbapenamase activity (44/57; 77.2%). This is expected since this phenotypic activity can be conferred by alternative mechanisms beyond the antibiotic resistance genes found on the BIOFIRE Pneumonia Panel. Specimens with unconfirmed (or unexpected) analytes were excluded from performance calculations for that particular analyte and BIOFIRE test results are reported separately.

The BIOFIRE Pneumonia Panel demonstrated positive percent agreement (PPA) of 100% with previous laboratory results for 11 of 14 analytes tested in BAL specimens (Table 55) and all of the analytes tested in sputum specimens (Table 56). The exceptions were *Klebsiella aerogenes*, *Proteus* spp. and RSV with a PPA of 50.0%, 80.0%, and 93.8% respectively, due to one false negative (FN) for each analyte. While PPA for most analytes was 100%, an insufficient number of specimens were tested for all but influenza A virus, parainfluenza virus, and respiratory syncytial virus in BAL. Contrived specimen testing was used to demonstrate performance for these analytes in an additional contrived study (see *Testing of Contrived Specimens*). Negative percent agreement (NPA) was 100% for all analytes except parainfluenza virus in BAL and influenza A virus in sputum; however, NPA was more thoroughly evaluated in the prospective clinical study.

Table 55. BIOFIRE Pneumonia Panel Archived BAL Specimen Performance Data Summary

Analyte	PPA			NPA		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Quantitative Bacteria						
Acinetobacter calcoaceticus-baumannii complex	4/4	100	51.0-100%	53/53	100	93.2-100%
Klebsiella aerogenes	1/2	50.0	-	55/55	100	93.5-100%
Proteus spp.	4/5	80.0	37.6-96.4%	48/48	100	92.6-100%
Serratia marcescens	10/10	100	72.2-100%	46/46	100	92.3-100%
Streptococcus pyogenes	1/1	100	-	57/57	100	93.7-100%
Antimicrobial Resistance Genes						
CTX-M	7/7	100	64.6-100%	29/29	100	88.3-100%
Atypical Bacteria						
Chlamydia pneumoniae	1/1	100	-	90/90	100	95.9-100%
Legionella pneumophila	1/1	100	-	57/57	100	93.7-100%
Viruses						
Adenovirus	8/8	100	67.6-100%	81/81	100	95.5-100%
Human metapneumovirus	11/11	100	74.1-100%	77/77	100	95.2-100%
Influenza A virus	21/21	100	84.5-100%	69/69	100	94.7-100%
Influenza B virus	3/3	100	43.9-100%	86/86	100	95.7-100%
Parainfluenza virus	17/17	100	81.6-100%	68/69	99.0	92.2-99.7%
Respiratory syncytial virus	15/16	93.8	71.7-98.9%	74/74	100	95.1-100%

Table 56. BIOFIRE Pneumonia Panel Archived Sputum Specimen Performance Data Summary

Analyte	PPA			NPA		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Quantitative Bacteria						
Streptococcus pyogenes	7/7	100	64.6-100%	8/8	100	67.6-100%
Antimicrobial Resistance Genes						
CTX-M	1/1	100	-	12/12	100	75.8-100%
Viruses						
Influenza A virus	2/2	100	34.2-100%	0/1	0	-

Testing of Contrived Specimens

A prospective clinical evaluation of the BIOFIRE Pneumonia Panel was performed during the 2016-2017 respiratory infection season at several geographically diverse clinical laboratories. Over 1600 specimens were analyzed from subjects whose specimens were submitted for microbial evaluation of lower respiratory tract pathogens. In the prospective study, some analytes were of insufficient prevalence to adequately demonstrate system performance and additional archived, preselected positive specimens containing rare analytes were also tested. Several analytes were so rare that both prospective and archived testing efforts were insufficient to demonstrate system performance. In this study, contrived clinical specimens were created to evaluate the sensitivity and specificity of the BIOFIRE Pneumonia Panel assays for these rare analytes (Table 57).

Table 57. Contrived Clinical Specimen Analytes

Analyte	Matrix	
	BAL	Sputum
Bacteria		

Analyte	Matrix	
	BAL	Sputum
Acinetobacter calcoaceticus-baumannii complex	X	
Klebsiella aerogenes	X	X
Klebsiella oxytoca	X	
Proteus spp.	X	
Serratia marcescens	X	
Streptococcus pyogenes	X	X
Atypical Bacteria		
Chlamydia pneumoniae	X	X
Legionella pneumophila	X	X
Mycoplasma pneumoniae	X	X
Viruses		
Adenovirus	X	X
Human metapneumovirus	X	X
Influenza A virus	X	X
Influenza B virus	X	X
Antibiotic Resistance Markers		
CTX-M	X	X
IMP	X	X
KPC	X	X
NDM	X	X
OXA-48-like	X	X
VIM	X	X

Contrived specimens (N=1125) were spiked using residual clinical samples that were pre-screened with the BIOFIRE Pneumonia Panel and found to be negative for the analytes of interest. Specimens were spiked with a variety of different isolates/strains for each organism at concentrations spanning observed ranges in clinical specimens. Different isolates of organisms were used from those used in analytical testing when possible. Samples positive for one analyte served as negatives for other analytes.

For the majority of analytes reported qualitatively, at least 25 of the contrived positive specimens had analyte concentrations at 2 × the limit of detection (LoD), while the remaining specimens were tested at additional concentrations that spanned clinically observed ranges. The “clinically observed range” was based on data from previous BIOFIRE Pneumonia Panel positive test results (e.g. observations from the prospective or archived studies). If a clinically observed range could not be determined for a particular analyte, specimens were spiked at various factors of LoD. If the stock concentration of organism did not allow for spiking at the highest level, the highest achievable level was used. For bacteria reported with binned values, specimens were spiked at various concentrations starting just below and then spanning the reported levels (i.e. 10^3 to ≥10^7 copies per milliliter (mL)).

Specimens were prepared and randomized at BIOFIRE such that the analyte status of each contrived specimen was unknown to the users performing the testing. BAL and sputum specimens were analyzed separately; however, the preparation and testing for both matrices were identical. Contrived specimens were frozen, then distributed to prospective study sites and tested according to the prospective clinical study protocol alongside clinical (non-contrived) specimens.

The positive percent agreement (PPA) and negative percent agreement (NPA) for the BIOFIRE Pneumonia Panel assays were determined using standard binomial sampling statistics. In this study, a success was defined as agreement between

the known composition of the contrived specimen and the BIOFIRE Pneumonia Panel result; i.e., a positive BIOFIRE Pneumonia Panel result for spiked samples (True Positive, TP) and a negative BIOFIRE Pneumonia Panel result for un-spiked samples (True Negative, TN).

The results of the 1125 specimens tested in this study are summarized in Table 58 for BAL and Table 59 for sputum below.

The majority of analytes in both specimen types met the performance goals of 90% PPA with an 80% lower bound of the 95% CI and 98% NPA with a 95% lower bound of the 95% CI. The exceptions being influenza A virus spiked into BAL and *Klebsiella aerogenes* spiked into BAL and sputum. Influenza A virus spiked in BAL demonstrated 86% PPA in part due to two missed detections at 0.2 × LoD and two additional missed detections at 2 × LoD from a strain that may have been under-quantified. However, BIOFIRE performance goals for influenza A virus in BAL were achieved in the archived study. *Klebsiella aerogenes* spiked in both sample types demonstrated 85.5% PPA in part due to five missed detections in BAL and four missed detections in sputum at a range of concentrations from a *Klebsiella aerogenes* strain that demonstrated poor reactivity with the BIOFIRE Pneumonia Panel.

Samples spiked with bacterial analytes just below the 10^4 copies/mL binned value (*i.e.,* near 1.00E+03), and samples spiked with other analytes below their LoD (*i.e.,* near 0.2 × LoD), produced the expected unreliable detection.

Table 58. BIOFIRE Pneumonia Panel Performance of Contrived BAL Specimens

Analyte	Sensitivity/PPA			Specificity/NPA		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Bacteria						
Acinetobacter calcoaceticus-baumannii complex	47/50	94.0	83.8-97.9%	598/598	100	99.4-100%
Klebsiella aerogenes[a]	47/55	85.5	73.8-92.4%	592/594	99.7	98.8-99.9%
Klebsiella oxytoca	46/50	92.0	81.2-96.8%	604/604	100	99.4-100%
Proteus spp.	48/50	96.0	86.5-98.9%	603/603	100	99.4-100%
Serratia marcescens	49/50	98.0	89.5-99.6%	604/604	100	99.4-100%
Streptococcus pyogenes	49/50	98.0	89.5-99.6%	597/597	100	99.4-100%
Atypical Bacteria						
Chlamydia pneumoniae	47/50	94.0	83.8-97.9%	604/604	100	99.4-100%
Legionella pneumophila	50/50	100	92.9-100%	599/599	100	99.4-100%
Mycoplasma pneumoniae	48/50	96.0	86.5-98.9%	603/604	99.8	99.1-100%
Viruses						
Adenovirus	50/53	94.3	84.6-98.1%	568/569	99.8	99.0-100%
Human metapneumovirus	50/50	100	92.9-100%	597/598	99.8	99.1-100%
Influenza A virus[b]	43/50	86.0	73.8-93.0%	585/585	100	99.3-100%
Influenza B virus[c]	47/50	94.0	83.8-97.9%	588/589	99.8	99.0-100%
Antibiotic Resistance Markers						
CTX-M	130/130	100	97.1-100%	323/324	99.7	98.3-100%
IMP	45/45	100	92.1-100%	412/412	100	99.1-100%
KPC	53/53	100	93.2-100%	400/400	100	99.1-100%
NDM	53/53	100	93.2-100%	404/404	100	99.1-100%
OXA-48-like	53/53	100	93.2-100%	307/307	100	98.8-100%
VIM	58/58	100	93.8-100%	399/399	100	99.0-100%

[a] Five FN specimens were spiked with an *K. aerogenes* (previously *E. aerogenes)* strain (ATCC 29751) that demonstrated poor reactivity with the BIOFIRE Pneumonia Panel (see Table 63).

[b] Two FN specimens were spiked with an influenza A virus strain that may have been under-quantified.

[c] Three FN specimens were spiked with an influenza B virus strain that may have been under-quantified.

Table 59. BIOFIRE Pneumonia Panel Performance of Contrived Sputum Specimens

Analyte	Sensitivity/PPA			Specificity/NPA		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Bacteria						
Klebsiella aerogenes[a]	47/55	85.5	73.8-92.4%	513/513	100	99.3-100%
Streptococcus pyogenes	48/50	96.0	86.5-98.9%	516/516	100	99.3-100%
Atypical Bacteria						
Chlamydia pneumoniae	49/50	98.0	89.5-99.6%	521/521	100	99.3-100%
Legionella pneumophila	50/50	100	92.9-100%	521/521	100	99.3-100%
Mycoplasma pneumoniae	48/50	96.0	86.5-98.9%	521/521	100	99.3-100%
Viruses						
Adenovirus	50/52	96.2	87.0-98.9%	494/494	100	99.2-100%
Human metapneumovirus	51/51	100	93.0-100%	520/520	100	99.3-100%
Influenza A virus[b]	47/50	94.0	83.8-97.9%	517/521	99.2	98.0-99.7%
Influenza B virus[c]	48/51	94.1	84.1-98.0%	516/517	99.8	98.9-100%
Antibiotic Resistance Markers						
CTX-M	121/122	99.2	95.6-99.9%	289/290	99.7	98.1-99.9%
IMP	43/44	97.7	88.2-99.6%	381/381	100	99.0-100%
KPC	54/54	100	93.4-100%	360/361	99.7	98.4-100%
NDM	53/53	100	93.2-100%	372/372	100	99.0-100%
OXA-48-like	51/51	100	93.0-100%	232/232	100	98.4-100%
VIM	56/56	100	93.6-100%	369/369	100	99.0-100%

[a] Four FN specimens were spiked with an *K. aerogenes* (previously *E. aerogenes)* strain (ATCC 29751) that demonstrated poor reactivity with the BIOFIRE Pneumonia Panel (see Table 63).

[b] Two FN specimens were spiked with an influenza A virus strain that may have been under-quantified.

[c] Two FN specimens were spiked with an influenza B virus strain that may have been under-quantified.

Testing of Polymicrobial Contrived Specimens

Additionally, two sets of individual BAL (N=60) and sputum (N=60) specimens were multi-spiked with randomized low, medium and high relative concentrations of either *A. baumannii*, *E. cloacae*, and *E. coli* or *K. oxytoca*, *P. mirabilis*, and *S. marcescens*. As shown in Table 60 and Table 61, the majority of the spiked organisms were reported at the expected relative low, medium, or high bin level by the BIOFIRE Pneumonia Panel. In four BAL specimens, *E. cloacae* was intended to be spiked at a medium level but was reported in a in a high (≥10^7) bin. Also, one specimen spiked with a high level of *P. mirabilis* was not detected (i.e. a false negative result for *P. mirabilis*).

Table 60. Polymicrobial Sputum Specimen Results

Organism Spiked Into Sputum					
		BIOFIRE Result			Total
		Low	Medium	High	
Spike Level	Low (10^4 copies/mL)	60	0	0	60
	Medium ($10^{5.5}$ copies/mL)	0	60	0	60
	High (10^7 copies/mL)	0	0	60	60

Table 61. Polymicrobial BAL Specimen Results

Organism Spiked Into BAL					
		BIOFIRE Result			Total
		Low	Medium	High	
Spike Level	Low (10^4 copies/mL)	60	0	0	60
	Medium ($10^{5.5}$ copies/mL)	0	56	4[a]	60
	High (10^7 copies/mL)	0	0	59	59[b]

[a] Quantified at the bin boundary and reported as>=10^7.

[b] One false negative result.

Limit of Detection

A limit of detection (LoD) was established for atypical bacteria and viruses detected by the BIOFIRE Pneumonia Panel. LoD was estimated by testing dilutions of contrived BAL or sputum samples containing known concentrations of organisms. Confirmation of LoD was achieved by testing at least 20 replicates per samples type on each BIOFIRE system (60 replicates total per sample type). LoD concentration was confirmed when the analyte was detected in at least 95% of the replicates tested.

The confirmed LoD for each atypical bacterium or virus (including a LoD for more than one isolate of the more genetically diverse viruses) is listed in Table 62. LoD concentration is based on quantification of each culture in viable units ($TCID_{50}$/mL or CFU/mL) and a corresponding molecular LoD concentration (DNA or RNA copies/mL) is provided based on quantitative real-time or digital PCR.

Table 62. Summary of Limit of Detection (LoD) for BIOFIRE Pneumonia Panel Atypical Bacteria and Viruses

Analyte	Isolate Strain/Serotype/Source ID	LoD Concentration[a]	
		Viable Units	Molecular (DNA or RNA)
Atypical Bacteria			
Chlamydia pneumoniae	**TW183** ATCC VR-2282	5.0E-01 $TCID_{50}$/mL[b]	3.3E+02 copies/mL[b]
Legionella pneumophila	**Philadelphia-1** ATCC 33152	5.0E+02 CFU/mL	1.6E+03 copies/mL
Mycoplasma pneumoniae	**M129** Zeptometrix 0801579	7.5E+01 $TCID_{50}$/mL[b]	3.5E+03 copies/mL[b]
Viruses			
Adenovirus	**Species A (A18)** ATCC VR-19	5.0E+01 $TCID_{50}$/mL	9.2E+03 copies/mL
	Species B (B3) Zeptometrix 0810062CF	1.0E+00 $TCID_{50}$/mL	1.8E+03 copies/mL
	Species C (C2) [c] ATCC VR-846	5.0E+00 $TCID_{50}$/mL	7.5E+03 copies/mL
	Species D (D37) Zeptometrix 0810119CF	2.5E-01 $TCID_{50}$/mL[b]	2.9E+03 copies/mL[b]
	Species E (E4) Zeptometrix 0810070CF	1.0E-01 $TCID_{50}$/mL[b]	3.5E+04 copies/mL[b]
	Species F (F41) ATCC VR-930	5.0E+00 $TCID_{50}$/mL	5.5E+03 copies/mL
Coronavirus	**229E** ATCC VR-740	5.0E-01 $TCID_{50}$/mL	8.1E+01 copies/mL
	HKU1 Clinical Specimen[d]	-	1.0E+04 copies/mL
	NL63 BEI NR-470	2.5E+00 $TCID_{50}$/mL[e]	5.4E+02 copies/mL[e]
	OC43 ATCC VR-759	5.0E+02 $TCID_{50}$/mL[e]	9.3E+03 copies/mL[e]
Human metapneumovirus	**16 Type A1**	5.0E+01 $TCID_{50}$/mL	5.9E+03 copies/mL

Analyte	Isolate Strain/Serotype/Source ID	LoD Concentration[a] Viable Units	LoD Concentration[a] Molecular (DNA or RNA)
	Zeptometrix 0810161CF		
Human rhinovirus/ enterovirus	**Rhinovirus Type 1A** Zeptometrix 810012CFN	1.5E+01 $TCID_{50}$/mL[b]	6.6E+03 copies/mL[b]
	Echovirus 6 Zeptometrix 0810076CF	1.0E+02 $TCID_{50}$/mL	5.7E+02 copies/mL
Influenza A virus	**H1N1pdm09 A/SwineNY/03/09** Zeptometrix 0810249CF	2.5E+00 $TCID_{50}$/mL[e]	1.7E+03 copies/mL[e]
	H3N2 A/Port Chalmers/1/73 ATCC VR-810	1.0E+00 $TCID_{50}$/mL[b]	2.1E+02 copies/mL[b]
Influenza B virus	**B/FL/04/06** Zeptometrix 0810255CF	5.0E+00 $TCID_{50}$/mL[e]	4.2E+02 copies/mL[e]
Parainfluenza virus	**Type 1** Zeptometrix 0810014CF	2.5E+01 $TCID_{50}$/mL	5.2E+03 copies/mL
	Type 2 Zeptometrix 0810015CF	2.5E+01 $TCID_{50}$/mL[e]	1.5E+03 copies/mL[e]
	Type 3 Zeptometrix 0810016CF	2.5E+01 $TCID_{50}$/mL[e]	3.8E+02 copies/mL[e]
	Type 4A Zeptometrix 0810060CF	2.5E+02 $TCID_{50}$/mL	8.1E+03 copies/mL
Respiratory syncytial virus	**Type A** Zeptometrix 0810040ACF	1.0E+00 $TCID_{50}$/mL	4.3E+02 copies/mL

[a] The listed concentration was confirmed with ≥95% detection on each BIOFIRE system in artificial BAL (aBAL) and/or sputum.

[b] LoD confirmation (≥95% detection) was achieved at a 2 to 5-fold lower concentration in aBAL.

[c] LoD for adenovirus species C is 10 – 100 x impaired when pouches are within 6 months of expiration (see Limitations).

[d] No cultured isolates of Coronavirus HKU1 were available for testing.

[e] LoD confirmation (≥95% detection) was achieved at a 2 to 5-fold lower concentration in sputum.

Note: LoD concentrations of the cultured viruses and the obligate intracellular atypical bacteria (*C. pneumoniae* and *M. pneumoniae*) are provided in units of $TCID_{50}$ (50% Tissue Culture Infectious Dose). $TCID_{50}$ is an indirect measure of viral or bacterial concentration based on infectivity and cytotoxicity and will therefore vary considerably depending on technique and methodology (including cell type, culture media and conditions, cytotoxicity of the virus, etc.). It is not appropriate to make determinations on relative sensitivity of different molecular assays for detection of viruses and bacteria based on LoD values measured in $TCID_{50}$/mL.

Note: LoD concentrations presented in copies/mL are based on extraction of nucleic acids from isolate cultures followed by independent quantitative real-time PCR assays (qPCR) or digital PCR. The accuracy of qPCR concentrations may be affected by extraction efficiency, standard curve accuracy, assay conditions, inhibitors, and/or sequence variation. The qPCR quantification has not been compared to reference material or other quantification methods.

No assay-specific LoD concentrations were determined for the bacterial analytes. For bacteria, the BIOFIRE Pneumonia Panel reports a Detected result when the estimated bacterial nucleic acid abundance is ≥10^3.5 copies/mL, and the panel reports a Not Detected result if there is no amplification or the estimated bacterial nucleic acid abundance is <10^3.5 copies/mL. Each assay was determined to be linear in relation to input concentration (slope ≈ 1.0 and coefficient of determination (Adj R^2) >0.95) and estimates of nucleic acid abundance and corresponding bin results were determined to be accurate within 0.5-log_{10} copies/mL when compared to a copies/mL input concentration determined by digital PCR.

No assay-specific LoD concentrations were determined for the antimicrobial resistance (AMR) gene assays. AMR genes are reported as Detected when an applicable bacterium is Detected and the assay for the AMR gene is positive. Positive AMR gene assay results were observed in ≥95% of 90 replicates when the applicable bacterium was tested at a concentration ≥10^3.5 copies/mL in the precision evaluation (see Precision (Reproducibility) below).

Analytical Reactivity (Inclusivity)

Analytical reactivity of BIOFIRE Pneumonia Panel assays was evaluated via a combination of empirical (wet) testing and *in silico* analysis of sequences available in public databases. Testing was performed on a collection of more than 350 genetically diverse viruses, bacteria, and antimicrobial resistance genes. The tested isolates represented relevant species, subspecies, strains, serotypes, or genotypes as well as temporal and geographic diversity for each of the panel analytes. Each isolate was tested in triplicate at concentrations near LoD or the lowest reportable level for the analyte. *In silico* analyses of sequence data was also used to make predictions of assay reactivity for less common strains or serotypes and AMR gene types that were not tested but that may be detected by the BIOFIRE Pneumonia Panel assays.

Atypical bacteria and viruses were tested and detected at concentrations within 3× LoD (Table 64 - Table 74). Bacteria were tested at a concentration of 1.0E+04 copies/mL (based on digital PCR of a single-copy gene in the bacterial chromosome) and the majority of isolates (94.4%) were detected with the expected bin result (Table 75 - Table 89) and when the bacterium was detected, the appropriate AMR gene(s) were also detected (Table 90 - Table 97).

Limitations on assay reactivity (based on wet testing observations) with specific viral and bacterial isolates or sequences and AMR gene types or sequences are noted in Table 63. Most limitations are associated with single-base sequence variants under one or more assay primers. Additional predicted limitations on reactivity based on *in silico* sequence analysis are provided in the footnotes and analyte-specific tables below.

Note: BIOFIRE Pneumonia Panel Influenza A virus and Influenza B virus assays are predicted to react with attenuated viruses used in vaccines.

Table 63. Limitations on Analytical Reactivity of BIOFIRE Pneumonia Panel Assays Observed in Testing

Limitation	Observed Result	Analyte	Strain/Isolate/Variant
Minor	**Detected** may be under-reported by one bin (≤10-fold)	*Enterobacter cloacae* complex	*Enterobacter hormaechei* (ATCC 49162)[a]
		Klebsiella pneumoniae group	*Klebsiella quasipneumoniae* subsp. *quasipneumoniae* (DSM 28211)[b]
		Moraxella catarrhalis	*Moraxella catarrhalis* (ATCC 23246)[c]
		Streptococcus pyogenes	*Streptococcus pyogenes* (ATCC 19615)
Major	**Detected** may be under-reported by two or more bins (>10-fold)	*Enterobacter cloacae* complex	*Enterobacter asburiae* (ATCC 35953, 35954, 35955, and 35957)[d]
		Klebsiella aerogenes	*Klebsiella (Enterobacter) aerogenes* (ATCC 29751)[e]
		mecA/C and MREJ (MRSA)	MREJ type xv[f]
	Not Detected	*Acinetobacter calcoaceticus-baumannii* complex	*Acinetobacter nosocomialis* (ATCC 700472)[g]
		Pseudomonas aeruginosa	*Pseudomonas aeruginosa* (ATCC 25619)[h]
		mecA/C and MREJ (MRSA)	MREJ type xviii[i] MREJ type xix[i] MREJ type xx[i]

[a] Minor limitation observed for this isolate due to sequence variant under a primer. Similar limitation predicted for two *E. hormaechei* sequences (3.0%) from public databases.

[b] Minor limitation observed for this isolate due to sequence variant under a primer. Similar limitation predicted for one *K. quasipneumoniae* sequence (20.0%) from public databases. Additional minor or major limitation predicted for four *K. pneumoniae* sequences (0.3%) from public databases.

[c] Minor limitation observed for this isolate. Additional minor or major limitation predicted for two *M. catarrhalis* sequences (3.3%) from public databases.

[d] Major limitation observed or predicted for these isolates due to sequence variance under primer. Similar limitation predicted for five *E. asburiae* sequences (10.9%), eight *E. cloacae* sequences (2.2%), and two *E. ludwigii* sequences (16.7%) from public databases.

[e] Major limitation observed for this isolate due to sequence variance under primers. Similar limitation predicted for six *K. aerogenes* sequences (4.3%) from public databases.

[f] Major limitation observed in testing and predicted by sequence analysis for approximately 40% of MREJ type xv-like sequences due to a variant base at the 3' end of an assay primer (March 2022).

[g] ATCC 700472 could not be confirmed as *A. nosocomialis* by sequence and may be a non-*Acinetobacter calcoaceticus-baumannii* complex species that has been mis-identified.

[h] Major limitation observed for this isolate due to sequence variant under a primer. Similar limitation predicted for one *P. aeruginosa* sequence (0.07%) from public databases.

[i] MREJ types xviii, xix and xx will not be detected. MREJ types xix and xx are described in association with methicillin-sensitive isolates, so the *mecA/C* and MREJ (MRSA) Not Detected result will be consistent with the methicillin-sensitive phenotype of isolates with these MREJ types.

Table 64. Adenovirus Isolates Tested and Detected

Organism	Species	Serotype [a]	Source [Strain/Location/Year]	Test Concentration		Result
				(copies/mL)	xLoD	
Adenovirus	A	18	ATCC VR-19 [Washington D.C./1954]	9.2E+03	1x	Adenovirus Detected
		12	ATCC VR-863 [Huie/Massachusetts]	2.7E+04	3x	
		31	Zeptometrix 0810073CF	2.7E+04	3x	
	B	3	Zeptometrix 0810062CF	1.8E+03	1x	
		7	ATCC VR-7 [Gomen/California/1954]	5.3E+03	3x	
		7A	Zeptometrix 0810021CF	5.3E+03	3x	
		7d/d2	Univ of Iowa Research Foundation [Iowa/2001]	5.3E+03	3x	
		7h	Univ of Iowa Research Foundation [Iowa/1999]	5.3E+03	3x	
		11	ATCC VR-12 [Slobitski/Massachusetts]	5.3E+03	3x	
		14	ATCC VR-15 [De Wit/Netherlands/1955]	5.3E+03	3x	
		16	ATCC VR-17 [CH.79/Saudi Arabia/1955]	5.3E+03	3x	
		21	ATCC VR-1833 [128/Saudi Arabia/1956]	5.3E+03	3x	
		34	ATCC VR-716 [Compton/1972]	5.3E+03	3x	
		35	ATCC VR-718 [Holden]	5.3E+03	3x	
		50	ATCC VR-1602 [Wan/Amsterdam/1988]	5.3E+03	3x	
	C	2	ATCC VR-846 [Adenoid 6]	7.5E+03	1x	
		1	Zeptometrix 0810050CF	2.3E+04	3x	
		5	Zeptometrix 0810020CF	2.3E+04	3x	
		6	ATCC VR-6 [Tonsil 99]	2.3E+04	3x	
	D	37	Zeptometrix 08100119CF	5.8E+02	1x	
		8	Zeptometrix 0810069CF	1.7E+03	3x	
		20	Zeptometrix 0810115CF	1.7E+03	3x	
	E	4	Zeptometrix 0810070CF	1.7E+04	1x	
		4	ATCC VR-1572 [RI-67/Missouri/1952-1953]	1.0E+04	0.6x	
		4a	Univ of Iowa Research Foundation [S Carolina/2004]	1.0E+04	0.6x	
	F	41	ATCC VR-930 [Tak 73-3544/ Netherlands/1973]	5.5E+03	1x	
		40	NCPV 0101141v	1.6E+04	3x	
		40	Zeptometrix 0810084CF	1.6E+04	3x	
		41	Zeptometrix 0810085CF	1.6E+04	3x	

[a] *In silico* analysis of available sequences predicts that the BIOFIRE Pneumonia Panel will also react with Adenovirus B55, C57, all species D serotypes, and G52.

Table 65. Coronavirus Isolates Tested and Detected

Organism	Type	Source [Location/Year]	Test Concentration		Result
			(copies/mL)	xLoD	
Coronavirus	229E	ATCC VR-740	8.1E+01	1x	Coronavirus Detected
		Zeptometrix 0810229CF	2.4E+02	3x	
	HKU1[a]	Clinical Specimen [Utah/2015]	1.0E+04	1x	
		Clinical Specimen [Detroit/2010]	3.0E+04	3x	
		Clinical Specimen [Utah/2015]	3.0E+04	3x	
		Clinical Specimen [Utah/2015]	3.0E+04	3x	
		Clinical Specimen [S Carolina/2010]	3.0E+04	3x	
	NL63	BEI NR-470[b] [Amsterdam/2003]	2.7E+02	1x	
		Zeptometrix 0810228CF	8.0E+02	3x	
	OC43	ATCC VR-759[c]	4.6E+03	1x	
		Zeptometrix 0810024CF	1.4E+04	3x	

[a] No cultured isolates of Coronavirus HKU1 were available for testing. Five clinical NPS specimens containing Coronavirus HKU1 were collected from different regions of the US in 2010 and 2015, quantified molecularly, and tested.

[b] Organism obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: Human Coronavirus NL63, NR-470.

[c] Discontinued part #. See ATCC VR-1558.

Table 66. Human Metapneumovirus Isolates Tested and Detected

Organism	Genotype	Serotype	Source [Location/Year]	Test Concentration		Result
				($TCID_{50}$/mL)	xLoD	
Human metapneumovirus	A1	16	Zeptometrix 0810161CF [Iowa10/2003]	5.0E+01	1x	Human Metapneumovirus Detected
		9	Zeptometrix 0810160CF [Iowa3/2002]	1.5E+02	3x	
	A2	20	Zeptometrix 0810163CF [Iowa14/2003]	1.5E+02	3x	
		27	Zeptometrix 0810164CF [Iowa27/2004]	1.5E+02	3x	
	B1	3	Zeptometrix0810156CF [Peru2/2002]	1.5E+02	3x	
		5	Zeptometrix 0810158CF [Peru3/2003]	1.5E+02	3x	
	B2	8	Zeptometrix 0810159CF [Peru6/2003]	1.5E+02	3x	
		4	Zeptometrix 0810157CF [Peru1/2002]	1.5E+02	3x	
		18	Zeptometrix 0810162CF [Iowa18/2003]	1.0E+02	3x	

Table 67. Human Rhinovirus and Enterovirus Isolates Tested and Detected

Species	Serotype	Source [Strain/Location/Year]	Test Concentration (copies/mL)	Test Concentration xLoD	Result
Human rhinovirus[a]					
A	1	Zeptometrix 0810012CFN [1A]	2.2E+03	1x	Human Rhinovirus/ Enterovirus Detected
	2	ATCC VR-482 [HGP]	1.7E+03	3x	
	7	ATCC VR-1601 [68-CV11]	1.7E+03	3x	
	16	ATCC VR-283 [11757/Washington DC/1960]	1.7E+03	3x	
	34	ATCC VR-507[b] [137-3]	1.7E+03	3x	
	57	ATCC VR-1600 [Ch47]	1.7E+03	3x	
	77	ATCC VR-1187 [130-63]	1.7E+03	3x	
	85	ATCC VR-1195 [50-525-CV54]	1.7E+03	3x	
B	3	ATCC VR-483 [FEB]	1.7E+03	3x	
	14	ATCC VR-284 [1059/S Carolina/1959]	1.7E+03	3x	
	17	ATCC VR-1663 [33342/N Carolina/1959]	1.7E+03	3x	
	27	ATCC VR-1137 [5870]	1.7E+03	3x	
	42	ATCC VR-338 [56822]	1.7E+03	3x	
	83	ATCC VR-1193 [Baylor 7]	1.7E+03	3x	
Enterovirus					
A	Coxsackievirus 10	ATCC VR-168 [NY/1950]	1.7E+03	3x	Human Rhinovirus/ Enterovirus Detected
	Enterovirus 71	ATCC VR-1432 [H]	1.7E+03	3x	
B	Coxsackievirus A9	Zeptometrix 0810017CF	1.7E+03	3x	
	Coxsackievirus B3	Zeptometrix 0810074CF	1.7E+03	3x	
	Coxsackievirus B4	Zeptometrix 0810075CF	1.7E+03	3x	
	Echovirus 6	Zeptometrix 0810076CF	5.7E+02	1x	
	Echovirus 9	Zeptometrix 0810077CF	1.7E+03	3x	
	Echovirus 11	Zeptometrix 0810023CF	1.7E+03	3x	
C	Coxsackievirus A21	ATCC VR-850 [Kuykendall/California/1952]	1.7E+03	3x	
	Coxsackievirus A24	ATCC VR-583 [DN-19/Texas/1963]	1.7E+03	3x	
D	Enterovirus 68	ATCC VR-1823 [US/MO/2014-18947]	1.7E+03	3x	

[a] The concentration used for Human Rhinovirus isolate testing was based on 3× the Enterovirus LoD concentration (5.7E+02 copies/mL).

[b] Discontinued part #; see ATCC VR-1365.

Table 68. Influenza A Virus Isolates Tested and Detected

Organism	Subtype	Source [Strain/Location/Year]	Test Concentration (copies/mL)	Test Concentration xLoD	Result
Influenza A virus	**Human**				Influenza A Detected
	H1N1	ATCC VR-219 [NWS/1933]	3.1E+02	3x	
		ATCC VR-95 [PR/8/1934a]	1.0E+03	1.5x[a]	
		ATCC VR-96 [Wiess/1943]	3.1E+02	3x	
		ATCC VR-97 [FM/1/1947]	3.1E+02	3x	
		ATCC VR-98 [Mal/302/1954]	3.1E+02	3x	
		ATCC VR-546 [Denver/1/1957]	3.1E+02	3x	
		Zeptometrix 0810036CF [New Caledonia/20/1999]	3.1E+02	3x	
		Zeptometrix 0810036CFN [Solomon Islands/3/2006]	3.1E+02	3x	
		Zeptometrix 0810244CF [Brisbane/59/2007]	3.1E+02	3x	
	H1N2	BEI NR-3478[b] [Kilbourne F63 A/NWS/1934 (HA) x A/Rockefeller Institute/5/1957 (NA)]	3.1E+02	3x	
	H1N1pdm09	Zeptometrix 0810249CF [SwineNY/03/2009]	6.6E+02	1x	
		Zeptometrix 0810109CFJ [Canada/6294/2009]	3.1E+02	3x	
		Zeptometrix 0810165CF [California/07/2009]	3.1E+02	3x	
		Zeptometrix 0810166CF [Mexico/4108/2009]	3.1E+02	3x	
		BEI NR-19823[c] [Netherlands/2629/2009]	3.1E+02	3x	
		BEI NR-42938[d] [Georgia/F32551/2012]	3.1E+02	3x	
		BEI NR-44345[e] [Hong Kong/H090-761-V1(0)/2009]	1.0E+03	1.5x[a]	
	H3N2	ATCC VR-810 [Port Chalmers/1/1973]	1.0E+02	1x	
		ATCC VR-776 [Alice (live attenuated vaccine)]	3.1E+02	3x	
		Zeptometrix 0810238CF [Texas /50/2012]	3.1E+02	3x	
		ATCC VR-547 [Aichi/2/1968]	3.1E+02	3x	
		ATCC VR-544 [Hong Kong/8/1968]	3.1E+02	3x	
		ATCC VR-822 [Victoria/3/1975]	3.1E+02	3x	
		Zeptometrix 0810252CF [Wisconsin/67/2005]	3.1E+02	3x	
		Zeptometrix 0810138CF [Brisbane/10/2007]	3.1E+02	3x	
	H3N2v	ODHL1 [Ohio/2012]	3.1E+02	3x	
	Avian				
	H2N2	BEI NR-2775[f] [Japan/305/1957]	3.1E+02	3x	

Organism	Subtype	Source [Strain/Location/Year]	Test Concentration (copies/mL)	Test Concentration xLoD	Result
	H2N3	MRIGlobal[g] [Mallard/Alberta/79/2003]	3.1E+02	3x	
	H5N1	MRIGlobal[g] [Chicken/Yunnan/1251/2003]	3.1E+02	3x	
	H5N2	MRIGlobal[g] [Northern pintail/Washinton/40964/2014]	3.1E+02	3x	
	H5N3	BEI NR-9682[h] [Duck/Singapore/645/97]	3.1E+02	3x	
	H5N8	MRIGlobal[g] [Gyrfalcon/Washing-ton/41088-6/2014]	3.1E+02	3x	
	H7N7	MRIGlobal[g] [Netherlands/219/2003]	3.1E+02	3x	
	H7N9	MRIGlobal[g] [Anhui/01/2013]	3.1E+02	3x	
	H10N7	BEI NR-2765[i] [Chicken/Germany/N/49]	3.1E+02	3x	
	Swine				
	H1N1 Swine	ATCC VR-333 [Swine/Iowa/15/1930]	3.1E+02	3x	
		ATCC VR-99 [Swine/1976/1931]	3.1E+02	3x	

[a] 1.5x the LoD for Influenza A virus H1N1pdm09 Zeptometrix 0810109CFN [SwineNY/03/2009] (6.6E+02 copies/mL).

[b] Genomic RNA obtained through BEI Resources NAID, NIH: Kilbourne F63: A/NWS/1934 (HA) x A/Rockefeller Institute/5/1957 (NA) (H1N2), Reassortant NWS-F, NR-9677.

[c] Virus obtained through BEI Resources, NIAID, NIH: Influenza A virus, A/Netherlands/2629/2009 (H1N1)pdm09, NR-19823.

[d] Virus obtained through BEI Resources, NIAID, NIH: Influenza A virus, A/Georgia/F32551/2012 (H1N1)pdm09, NR-42938.

[e] Virus obtained through BEI Resources, NIAID, NIH: Influenza A virus, A/Hong Kong/H090-761-V1(0)/2009 (H1N1)pdm09, NR-44345.

[f] Genomic RNA obtained through BEI Resources, NIAID, NIH: Genomic RNA from Influenza A virus, A/Japan/305/1957 (H2N2), NR-2775.

[g] Isolate provided and tested by MRI Global, Kansas City, MO.

[h] Genomic RNA obtained through BEI Resources, NIAID, NIH: Genomic RNA from Influenza A virus, A/duck/Singapore/645/1997 (H5N3), Wild Type, NR-9682.

[i] Genomic RNA obtained through BEI Resources, NIAID, NIH: Genomic RNA from Influenza A virus, A/chicken/Germany/N/1949 (H10N7), NR-2765.

Table 69. Influenza B Virus Isolates Tested and Detected

Organism	Lineage	[Strain/Location/Year], Source	Test Concentration (copies/mL)	Test Concentration xLoD	Result
Influenza B virus	N/A	ATCC VR-101 [Lee/1940]	6.3E+02	3x	Influenza B Detected
		ATCC VR-102 [Allen/1945]	6.3E+02	3x	
		ATCC VR-103 [GL/1739/1954]	6.3E+02	3x	
		ATCC VR-296 [1/Maryland/1959]	6.3E+02	3x	
		ATCC VR-295 [2/Taiwan/1962]	6.3E+02	3x	
		ATCC VR-786 [Brigit/Russia/1969]	6.3E+02	3x	
	Victoria	ATCC VR-823 [5/Hong Kong/1972]	6.3E+02	3x	
		Zeptometrix 0810258CF [2506/Malaysia/2004]	6.3E+02	3x	
		CDC 2005743348 [1/Ohio/2005]	6.3E+02	3x	
	Yamagata	Zeptometrix 0810256CF [07/Florida/2004]	6.3E+02	3x	
		Zeptometrix 0810255CF [04/Florida/2006]	2.1E+02	1x	
		Zeptometrix 0810241CF [1/Wisconsin/2010]	6.3E+02	3x	
		Zeptometrix 0810239CF [2/Massachusetts/2012]	6.3E+02	3x	

Table 70. Parainfluenza Virus Isolates Tested and Detected

Organism	Type	Source [Strain/Location/Year]	Test Concentration (copies/mL)	Test Concentration xLoD	Result
Parainfluenza virus	1	Zeptometrix 0810014CF	5.2E+03	1x	Parainfluenza Virus Detected
		BEI NR-48680[a] [FRA/29221106/2009]	1.6E+04	3x	
		ATCC VR-94 [C-35/Washington DC/1957]	1.6E+04	3x	
	2	Zeptometrix 0810015CF	1.5E+03	1x	
		ATCC VR-92 [Greer/Ohio/1955]	8.9E+02	0.6x	
	3	Zeptometrix 0810016CF	1.9E+02	1x	
		BEI NR-3233[b] [NIH 47885, Wash/47885/57]	5.7E+02	3x	
		ATCC VR-93 [C-243/Washington DC/1957]	5.7E+02	3x	
	4	Zeptometrix 0810060CF	8.1E+03	1x	
		ATCC VR-1378 [M-25/1958]	2.4E+04	3x	
		Zeptometrix 0810060BCF	2.4E+04	3x	
		ATCC VR-1377 [CH-19503/Washington DC/1962]	2.4E+04	3x	

[a] Virus obtained through BEI Resources, NIAID, NIH: Human Parainfluenza virus 1, HPIV1/FRA/29221106/2009, NR-48680.

[b] Virus obtained through BEI Resources, NIAID, NIH: Human Parainfluenza virus 3, NIH 47885, NR-3233.

Table 71. Respiratory Syncytial Virus Isolates Tested and Detected

Organism	Type	Source [Strain/Location/Year]	Test Concentration (copies/mL)	Test Concentration xLoD	Result
Respiratory syncytial virus	A	Zeptometrix 0810040ACF [2006]	4.3E+02	1x	Respiratory Syncytial Virus Detected
		ATCC VR-26 [Long/Maryland/1956]	1.3E+03	3x	
		ATCC VR-1540 [A2/Melbourne/1961]	1.3E+03	3x	
	B	Zeptometrix 0810040CF [Ch-93 (18)-18]	1.3E+03	3x	

Organism	Type	Source [Strain/Location/Year]	Test Concentration		Result
			(copies/mL)	xLoD	
		ATCC VR-1400 [WV/14617/1985]	1.3E+03	3x	
		ATCC VR-955 [9320/Massachusetts/1977]	1.3E+03	3x	
		ATCC VR-1580 [18537/Washington DC/1962]	1.3E+03	3x	

Table 72. *Chlamydia pneumoniae* Isolates Tested and Detected

Organism	Source [Strain]	Test Concentration		Result
		(copies/mL)	xLoD	
Chlamydia pneumoniae	ATCC VR-2282 [TW-183/Taiwan/1965]	6.7E+01	1x	*Chlamydia pneumoniae* Detected
	ATCC VR-1310 [CWL-029]	2.0E+02	3x	
	ATCC VR-1360 [CM-1/Georgia]	2.0E+02	3x	
	ATCC 53592 [AR-39/Seattle/1983]	2.0E+02	3x	

Table 73. *Legionella pneumophila* Isolates Tested and Detected

Species/Subspecies	Serogroup	Source [Strain]	Test Concentration		Result
			(CFU/mL)	xLoD	
L. pneumophila	1	ATCC 33152 [Philadelphia-1]	5.0E+02	1x	*Legionella pneumophila* Detected
	3	ATCC 33155 [Bloomington-2]	1.5E+03	3x	
L. pneumophila subsp. *fraseri*	4	ATCC 33156 [Los Angeles-1]	1.5E+03	3x	
	5	ATCC 33216 [Dallas 1E]	1.5E+03	3x	
L. pneumophila subsp. *pascullei*	5	ATCC 33737 [U8W]	1.5E+03	3x	
L. pneumophila subsp. *pneumophila*	10	ATCC 43283 [Leiden 1]	1.5E+03	3x	
	14	ATCC 43703 [1169-MN-H]	1.5E+03	3x	

Table 74. *Mycoplasma pneumoniae* Isolates Tested and Detected

Organism	Type	Source [Strain]	Test Concentration		Result
			(copies/mL)	xLoD	
Mycoplasma pneumoniae	1	Zeptometrix 0801579 [M129]	1.2E+03	1x	*Mycoplasma pneumoniae* Detected
		ATCC 29342 [M129-B7]	3.5E+03	3x	
		ATCC 29085 [PI 1428]	3.5E+03	3x	
	2	ATCC 15531-TTR [FH strain of Eaton Agent [NCTC 10119]]	3.5E+03	3x	
		ATCC 15492 [Mac]	3.5E+03	3x	
	unknown	ATCC 15293 [M52]	3.5E+03	3x	
		ATCC 15377 [Bru]	3.5E+03	3x	
		ATCC 39505 [Mutant 22]	3.5E+03	3x	
		ATCC 49894 [UTMB-10P]	3.5E+03	3x	

Table 75. *Acinetobacter calcoaceticus-baumannii* complex Isolates Tested and Detected

Organism	Source [Strain]	Test concentration (copies/mL)	Result
A. baumannii	ATCC 9955 [6-561]	1.0E+04	*Acinetobacter calcoaceticus-baumannii* complex Detected
	ATCC 19606 [2208 Type strain]	1.0E+04	
	ATCC 17961 [CDC 7788]	1.0E+04	
	AR-Bank #0033	1.0E+04	
	GRE 1153064	1.0E+04	
	GRE 1062081	1.0E+04	
A. calcoaceticus	ATCC 51432	1.0E+04	
	ATCC 23055 [46]	1.0E+04	
	ATCC 14987 [HO-1]	1.0E+04	
A. calcoaceticus subsp. *anitratus*	ATCC 15308 [NCTC 7844]	1.0E+04	
A. pittii	ATCC 19004 [57.071.228]	1.0E+04	
A. nosocomialis [a]	ATCC 17903 [NCTC 8102]	1.0E+04	
A.seifertii	CCUG 34785	1.0E+04	

[a] *A. nosocomialis* ATCC 700472 was not detected at any concentration. Sequencing suggests the isolate may be mis-identified.

Table 76. *Enterobacter cloacae* complex Isolates Tested and Detected

Organism	Source [Strain]	Test concentration (copies/mL)	Result
E. cloacae	ATCC 49141 [AmMs 204]	1.0E+04	*Enterobacter cloacae* complex Detected
	ATCC BAA-1143 [Entb 55M]	1.0E+04	
	ATCC BAA-2341 [1101152]	1.0E+04	
	AR-Bank #0154	1.0E+04	
	NCTC 13464	1.0E+04	
E. cloacae subsp. *cloacae*	ATCC 13047 [Type Strain]	1.0E+04	
E. cloacae subsp. *dissolvens*	ATCC 23373D[a] [ICPB ED105]	1.0E+04	
E. asburiae	ATCC 35953[b] [CDC 1497-78 Type Strain]	1.0E+06[b]	
	ATCC 35957[b] [CDC 570-83]	1.0E+06[b]	

	CCUG 59410[c]	1.0E+04	
E. hormaechei	ATCC 49162[b] [CDC 992-77]	1.0E+05[b]	
	ATCC BAA-2082	1.0E+04	
E. kobei	*In silico* prediction (not tested)		
E. ludwigii	CCUG 23050	1.0E+04	
E. mori	*In silico* prediction (not tested)		
E. roggenkampii	*In silico* prediction (not tested)		

[a] Genomic DNA from *E. cloacae* subsp. *dissolvens*.

[b] See Table 63 for limitation.

[c] Isolate was previously described as *Enterobacter kobei*.

Table 77. *Escherichia coli* Isolates Tested and Detected

Organism	Source [Strain]	Test concentration (copies/mL)	Result
E. coli	ATCC 25922 [FDA strain Seattle 1946]	1.0E+04	*Escherichia coli* Detected
	ATCC 43888 [CDC B568-73]	1.0E+04	
	AR-Bank #0061	1.0E+04	
	AR-Bank #0086	1.0E+04	
	AR-Bank #0137	1.0E+04	
	AR-Bank #0150	1.0E+04	
	AR-Bank #0162	1.0E+04	
	GRE 1062016	1.0E+04	
	GRE 1252008	1.0E+04	
	GRE 1252009	1.0E+04	
	GRE 1256018	1.0E+04	
	Zeptometrix 0801905 [Z136]	1.0E+04	
	ATCC 29930 [WRAIR I virulent]	1.0E+04	

Table 78. *Haemophilus influenzae* Isolates Tested and Detected

Organism	Serotype	Source [Strain/Location/Year]	Test concentration (copies/mL)	Result
H. influenzae	Type a	ATCC 9006 [AMC 36-A-3 [610, PCM 2436]]	1.0E+04	*Haemophilus influenzae* Detected
	Type b	ATCC 10211 [AMC 36-A-1 [572]], Biotype 1	1.0E+04	
	Type c	ATCC 49699 [C 9007]	1.0E+04	
	Type d	ATCC 9008 [AMC 36-A-6 [611]]	1.0E+04	
	Type e	ATCC 8142 [AMC 36-A-7 [595, NCTC 8472]]	1.0E+04	
	Type f	ATCC 700223 [GA1264]	1.0E+04	
	Biogroup aegyptius	ATCC 11116 [180-a [NCTC 8502]]	1.0E+04	
	Non-typeable	ATCC 51907 [Rd [KW20]]	1.0E+04	

NOTE: The Hinfluenzae assay will not react with strains that do not carry the hpd gene.[132]

Table 79. *Klebsiella* (*Enterobacter*) *aerogenes* Isolates Tested and Detected

Organism	Source [Strain]	Test concentration (copies/mL)	Result
K. aerogenes	ATCC 13048 [NCTC 10006]	1.0E+04	*Klebsiella aerogenes* Detected
	AR-Bank #0062	1.0E+04	
	AR-Bank #0074	1.0E+04	
	AR-Bank #0161	1.0E+04	
	GRE 1254066	1.0E+04	
	ATCC 29751[a] [MULB-250]	1.0E+07[a]	

[a] Previously known as *Enterobacter aerogenes*.

[b] See Table 63 for limitation.

Table 80. *Klebsiella oxytoca* Isolates Tested and Detected

Organism	Source [Strain]	Test concentration (copies/mL)	Result
K. oxytoca	ATCC 13182 [479-2 Type strain]	1.0E+04	*Klebsiella oxytoca* Detected
	ATCC 43086 [Pasco 201]	1.0E+04	
	ATCC 49131 [AmMS 101]	1.0E+04	
	ATCC 700324 [LBM 90.11.033]	1.0E+04	
	ATCC 8724 [NRRL B-199]	1.0E+04	
	AR-Bank #0147	1.0E+04	
	JMI 2523	1.0E+04	
	JMI 2661	1.0E+04	
	JMI 7818	1.0E+04	
	JMI 10678	1.0E+04	
	JMI 14611	1.0E+04	
	GRE 1254054	1.0E+04	

Table 81. *Klebsiella pneumoniae* Group Isolates Tested and Detected

Organism	Source [Strain]	Test concentration (copies/mL)	Result
K. pneumoniae	ATCC BAA-1705 [ART 2008133]	1.0E+04	*Klebsiella pneumoniae* Detected
	AR-Bank #0068	1.0E+04	
	AR-Bank #0075	1.0E+04	
	AR-Bank #0076	1.0E+04	
	AR-Bank #0079	1.0E+04	
	AR-Bank #0080	1.0E+04	
	AR-Bank # 0097	1.0E+04	
	AR-Bank #0107	1.0E+04	
	AR-Bank #0153	1.0E+04	
	GRE 1062084	1.0E+04	
	GRE 1355030	1.0E+04	
	JMI 328	1.0E+04	
	JMI 766	1.0E+04	
	NCTC 13465	1.0E+04	
	Zeptometrix 0801886	1.0E+04	
K. pneumoniae subsp. *ozaenae*	ATCC 11296 [AMC 35-E-5]	1.0E+04	
K. pneumoniae subsp. *pneumoniae*	ATCC 13883 [NCTC 9633]	1.0E+04	
K. pneumoniae subsp. *rhinoscleromatis*	ATCC 13884 [NCTC 5046]	1.0E+04	
K. quasipneumoniae subsp. *quasipneumoniae*	DSM 28211[a] [01A030, SB11]	1.0E+05[a]	
K. quasipneumoniae subsp. *simipneumoniae*	DSM 28212 [07A044, SB30]	1.0E+04	
K. variicola	ATCC BAA-830 [F2R9]	1.0E+04	

[a] See Table 63 for limitation.

Table 82. *Moraxella catarrhalis* Isolates Tested and Detected

Organism	Source [Strain]	Test concentration (copies/mL)	Result
M. catarrhalis	ATCC 25238 [Ne 11]	1.0E+04	*Moraxella catarrhalis* Detected
	ATCC 25240 [N9]	1.0E+04	
	ATCC 8176 [20]	1.0E+04	
	ATCC 23246 [a] [NCTC 4103]	1.0E+05[a]	
	ATCC 49143 [Am MS 116]	1.0E+04	

[a] See Table 63 for limitation.

Table 83. *Proteus* spp. Isolates Tested and Detected

Organism	Source [Strain]	Test concentration (copies/mL)	Result
P. mirabilis	ATCC 29906 [1003]	1.0E+04	*Proteus* spp. Detected
	ATCC 33583 [571101]	1.0E+04	
	ATCC 35659 [LRA 08 01 73]	1.0E+04	
	AR-Bank #0156	1.0E+04	
	AR-Bank #0159	1.0E+04	
	GRE 1254053	1.0E+04	
P. hauseri	ATCC 13315 [NCTC 4175 Strain Lehmann]	1.0E+04	
	ATCC 700826 [CDC 1732-80]	1.0E+04	
P. penneri	ATCC 33519 [Type Strain CDC 1808-73]	1.0E+04	
	ATCC 35197 [CDC 1655-67]	1.0E+04	
P. vulgaris	ATCC 29905	1.0E+04	
	ATCC 33420	1.0E+04	
	ATCC 27973 [CDC 1787-64-SC1]	1.0E+04	

Table 84. *Pseudomonas aeruginosa* Isolates Tested and Detected

Organism	Source [Strain]	Test concentration (copies/mL)	Result
P. aeruginosa[a]	ATCC 10145 [MDB strain BU 277 type strain]	1.0E+04	*Pseudomonas aeruginosa* Detected
	ATCC BAA-1744 [109246]	1.0E+04	
	ATCC 19429 [NCTC 6750]	1.0E+04	
	ATCC 27853 [Boston 41501]	1.0E+04	
	AR-Bank #0054	1.0E+04	
	AR-Bank #0092	1.0E+04	
	AR-Bank #0100	1.0E+04	
	AR-Bank #0103	1.0E+04	
	AR-Bank #0111	1.0E+04	
	Creighton University PS28	1.0E+04	
	NCTC 13437	1.0E+04	

[a] *P. aeruginosa* ATCC 25619 was not detected at any concentration tested. See Table 63 for limitation.

Table 85. *Serratia marcescens* Isolates Tested and Detected

Organism	Source [Strain]	Test concentration (copies/mL)	Result
S. marcescens	ATCC 13880 [Type strain]	1.0E+04	*Serratia marcescens* Detected
	ATCC 27137 [CDC 3100-71]	1.0E+04	
	ATCC 43297 [3G]	1.0E+04	
	ATCC BAA-885 [Type strain KRED]	1.0E+04	
	GRE 1659005	1.0E+04	
	GRE 1659004	1.0E+04	
	JMI 697	1.0E+04	

Table 86. *Staphylococcus aureus* Isolates Tested and Detected

Organism	Source [Strain] [PFGE Type if applicable]	Test concentration (copies/mL)	Result
S. aureus	***Staphylococcus aureus* representing PFGE Types USA100-USA1200**		*Staphylococcus aureus* Detected
	NARSA NRS705 [PFGE USA100]	1.0E+04	
	NARSA NRS701 [PFGE USA200]	1.0E+04	
	ATCC BAA-1717 [PFGE USA300]	1.0E+05[a]	
	NARSA NRS683 [PFGE USA300]	1.0E+04	
	NARSA NRS662 [PFGE USA300]	1.0E+04	
	NARSA NRS707 [PFGE USA300]	1.0E+04	
	ATCC BAA-1707 [PFGE USA400]	1.0E+04	
	NARSA NRS691 [PFGE USA500]	1.0E+04	
	NARSA NRS648 [PFGE USA600]	1.0E+04	
	NARSA NRS689 [PFGE USA700]	1.0E+04	
	NARSA NRS668 [PFGE USA800]	1.0E+04	
	ATCC BAA-1749 [PFGE USA900 96:308]	1.0E+04	
	ATCC BAA-1759 [PFGE USA900 N7129]	1.0E+04	
	ATCC BAA-1700 [PFGE USA1000]	1.0E+04	
	BEI NR-46081 [PFGE USA1100 HIP12899]	1.0E+04	
	ATCC BAA-1765 [PFGE USA1200 102-04]	1.0E+04	
	ATCC BAA-1691 [Not USA100-1100]	1.0E+04	
	Methicillin Sensitive *Staphylococcus aureus* (MSSA)		
	ATCC 10832 [Wood 46]	1.0E+04	
	ATCC 14154 [Rose]	1.0E+04	
	ATCC 12600 [NCTC Type strain]	1.0E+04	
	ATCC 25923 [Seattle/1945]	1.0E+04	
	ATCC 29213 [Wichita]	1.0E+04	
	ATCC BAA-2421 [Mass/2010]	1.0E+04	
	Rennes 1060728	1.0E+04	
	GRE 1062519 [SCC*mec* Type: III / MREJ xix][b]	1.0E+04	
	Borderline Resistant *Staphylococcus aureus* (BORSA)		
	SUN1 [Sunnybrook]	1.0E+04	
	Methicillin Resistant *Staphylococcus aureus* (MRSA)		
	ATCC 43300 [F182 Kansas / SCC*mec* Type: II]	1.0E+04	
	ATCC BAA-2422 [Worcester MA/2010 / SCC*mec* Type: II]	1.0E+04	
	ATCC BAA-1720 [MRSA252 / SCC*mec* Type: II / PFGE USA200]	1.0E+04	
	NARSA NRS745 [CA-629 / SCC*mec* Type: V]	1.0E+04	
	ATCC BAA-38 [E2125 / SCC*mec* Type: I]	1.0E+04	
	NARSA NRS686 [MREJ type i]	1.0E+04	
	ATCC BAA-44 [HPV107 / SCC*mec* Type: I / PFGE: Iberian]	1.0E+04	
	ATCC BAA-41 [NYBK2464 / SCC*mec* Type: II / PFGE 100]	1.0E+04	
	NARSA NRS385 [MREJ type ii]	1.0E+04	
	ATCC BAA-42 [HDE288 / SCCmec: Type VI / PFGE 800]	1.0E+04	
	ATCC BAA-39 [HUSA304 / SCC*mec* Type: III]	1.0E+04	
	ATCC BAA-40 [CPS22 / SCC*mec* Type: III]	1.0E+04	
	GRE 1062264 [SCC*mec* Type: IV / MREJ type iv]	1.0E+04	
	GRE 1055015 [SCC*mec* Type: IVa / MREJ type vi]	1.0E+04	
	GRE 0759084 [SCC*mec* Type: IV / MREJ type v]	1.0E+04	
	GRE 0860042 [SCC*mec* Type: III / MREJ type vii]	1.0E+04	
	GRE 1052034 [MREJ ix]	1.0E+04	
	GRE 1151100 [SCC*mec* Type: IV / MREJ type xi]	1.0E+04	
	GRE 0960006 [MREJ type xii]	1.0E+04	
	GRE 1055017 [SCC*mec* Type: IVa / MREJ type xiii]	1.0E+04	
	GRE 0759163 [MREJ type xiv]	1.0E+04	

Organism	Source [Strain] [PFGE Type if applicable]	Test concentration (copies/mL)	Result
	GRE 1062373 [MREJ type xv]	1.0E+04	
	GRE 1057114 [MREJ type xvii]	1.0E+04	
	GRE 1062292 [MREJ type xviii]	1.0E+04	
	Methicillin Resistant Staphylococcus aureus (MRSA) - *mecC*+		
	ATCC BAA-2312 [M10/0061 / SCC*mec* Type: XI / *mecC*]	1.0E+04	
	ATCC BAA-2313 [M10/0148 / SCC*mec* Type: XI / *mecC*]	1.0E+04	

[a] *Staphylococcus aureus* ATCC BAA-1717 was not detected at 1.0E+04 copies/mL but was detected at 1.0E+05 copies/mL with accurate bin results. The expected detection and 10^4 bin result was reported in ≥50% of additional replicates tested at 1.0E+04 copies/mL and no limitation on reactivity could be identified based on the isolate sequence.

[b] MREJ type xix characterized as MSSA.[133]

Table 87. *Streptococcus agalactiae* Isolates Tested and Detected

Organism	Source [Strain]	Test concentration (copies/mL)	Result
S. agalactiae	NCTC 8017 [MK 104 P]	1.0E+04	*Streptococcus agalactiae* Detected
	ATCC 13813 [Ia/c Type Strain]	1.0E+04	
	ATCC 12403 [III Typing Strain D136C]	1.0E+04	
	ATCC 12386 [Grouping strain O90R]	1.0E+04	
	ATCC BAA-611 [V 2603 V/R]	1.0E+04	
	ATCC BAA-2669 [VIII 5030-08]	1.0E+04	
	Clinical Isolate [Utah/2010/CI03]	1.0E+04	

Table 88. *Streptococcus pneumoniae* Isolates Tested and Detected

Organism	Serotype	Source [Strain]	Test concentration (copies/mL)	Result
S. pneumoniae	3	ATCC 6303	1.0E+04	*Streptococcus pneumoniae* Detected
	5	ATCC BAA-341 [SPN1439-106]	1.0E+04	
	11A	NCTC 11900 [Gorman]	1.0E+04	
	14	ATCC 700672 [VH14]	1.0E+04	
	19A	ATCC 700673 [Hungary 19A-6]	1.0E+04	
	Non-capsulated	ATCC BAA-255 [R6]	1.0E+04	
	unknown	ATCC BAA-1409 [62076]	1.0E+04	

Table 89. *Streptococcus pyogenes* Isolates Tested and Detected

Organism	Source [Strain]	Test concentration (copies/mL)	Result
S. pyogenes	ATCC 12344 [Typing strain T1, NCIB 11841, SF 130]	1.0E+04	*Streptococcus pyogenes* Detected
	ATCC 12348 [Typing strain S43 Type 6]	1.0E+04	
	ATCC 12384 [Typing strain C203 Type 3]	1.0E+04	
	ATCC 19615[a] [Bruno]	1.0E+06[a]	
	ATCC 700294 [SF370; M1 GAS [M-type 1 T-type 1]]	1.0E+05[b]	
	ATCC 49399 [QC A62]	1.0E+04	
	ATCC BAA-595 [MGAS 315, serotype M3]	1.0E+04	
	ATCC BAA-947 [MGAS 5005, serotype M1]	1.0E+04	

[a] See Table 63 for limitation.

[b] *Streptococcus pyogenes* ATCC 700294 was detected in 3/5 replicates at 1.0E+04 copies/mL with 10^4 copies/mL bin results and 3/3 replicates at 1.0E+05 copies/mL with 10^5 copies/mL bin results. The expected detection and 10^4 bin result was reported in ≥50% of additional replicates tested at 1.0E+04 copies/mL and no limitation on reactivity could be identified based on isolate sequence.

The following tables (Table 90 through Table 97) describe the reactivity of the AMR genes assays with different AMR gene types in various host bacteria. Results are shown for the isolates tested as well as predictions of reactivity with untested AMR gene types based on *in silico* analysis of sequences retrieved from public databases from June 2016 to Sept 2016.

Table 90. Isolates Containing *mecA/C* and MREJ Tested and Detected

Organism	Source [Strain]	Test concentration (copies/mL)	Result
S. aureus	**Methicillin Sensitive *Staphylococcus aureus* (MSSA) containing SCC*mec* cassette (non-functional *mecA* variant)**		*mecA/C* and MREJ Detected
	ATCC BAA-2421 [Mass/2010]	1.0E+04	
	Methicillin Resistant *Staphylococcus aureus* (MRSA) (Characterized SCC*mec* Types)		
	NARSA NRS705 [NY-12 / SCC*mec* Type: II]	1.0E+04	
	NARSA NRS701 [MN-082 / SCC*mec* Type: II]	1.0E+04	
	ATCC BAA-1717 [TCH1516 / SCC*mec* Type: IVa]	1.0E+05[a]	
	NARSA NRS683 [GA-298 / SCC*mec* Type: IV]	1.0E+04	

Organism	Source [Strain]	Test concentration (copies/mL)	Result
	NARSA NRS662 [CO-34 / SCC*mec* Type: IV]	1.0E+04	
	NARSA NRS707 [NY-155 / SCC*mec* Type: IV]	1.0E+04	
	ATCC BAA-1707 [MW2 /SCC*mec* Type: IV]	1.0E+04	
	NARSA NRS691 [GA-62 /SCC*mec* Type: IV]	1.0E+04	
	NARSA NRS648 [CA-347 /SCC*mec* Type:II or IV]	1.0E+05[a]	
	NARSA NRS689 [GA-442 / SCC*mec* Type: IV]	1.0E+04	
	NARSA NRS668 [CO-72 / SCC*mec* Type: IV]	1.0E+04	
	ATCC BAA-1700 [HFH-33798 / SCC*mec* Type: IVb]	1.0E+04	
	BEI NR-46081[b] (NRSA NRS484) [HIP12899 / SCCmec Type: IV]	1.0E+05[a]	
	ATCC BAA-1691 [HFH-30137 / SCC*mec* Type: IV]	1.0E+04	
	ATCC 43300 [F182 Kansas / SCC*mec* Type: II]	1.0E+04	
	ATCC BAA-2422 [Worcester MA/2010 / SCC*mec* Type: II]	1.0E+04	
	ATCC BAA-1720 [MRSA252 / SCC*mec* Type: II]	1.0E+04	
	NARSA NRS745 [CA-629 / SCC*mec* Type: IV or V]	1.0E+04	
	Methicillin Resistant *Staphylococcus aureus* (MRSA) (Characterized MREJ Types)		
	ATCC BAA-38 [MREJ type i]	1.0E+04	
	NARSA NRS686 [MREJ type i]	1.0E+04	
	ATCC BAA-44 [MREJ type ii]	1.0E+04	
	ATCC BAA-41 [MREJ type ii]	1.0E+04	
	NARSA NRS385 [MREJ type ii]	1.0E+04	
	ATCC BAA-42 [MREJ type ii]	1.0E+04	
	ATCC BAA-39 [MREJ type iii]	1.0E+04	
	ATCC BAA-40 [MREJ type iv]	1.0E+04	
	GRE 1062264 [MREJ type iv]	1.0E+04	
	GRE 1055015 [MREJ type vi]	1.0E+04	
	GRE 0860042 [MREJ type vii]	1.0E+04	
	GRE 1052034 [MREJ type ix]	1.0E+04	
	GRE 1151100 [MREJ type xi]	1.0E+04	
	GRE 0960006 [MREJ type xii]	1.0E+04	
	GRE 1055017 [MREJ type xiii]	1.0E+04	
	GRE 0759163 [MREJ type xiv]	1.0E+04	
	GRE 1062373 [MREJ type xv][c]	1.0E+06[c]	
	GRE 1057114 [MREJ type xvii]	1.0E+04	
	GRE 1062292 [MREJ type xviii][c]	3.3E+08[c]	*mecA/C* and MREJ (MRSA) Not Detected
	GRE 1062519 [MREJ type xix][c,d]	1.0E+07[c]	
	Methicillin Resistant *Staphylococcus aureus* (MRSA) (SCC*mec* Type: XI / *mec*C / *mecA*$_{LGA251}$ variants)		
	ATCC BAA-2312 [M10/0061 / SCC*mec* Type: XI / *mecC*]	1.0E+04	*mecA/C* and MREJ (MRSA) Detected
	ATCC BAA-2313 [M10/0148 / SCC*mec*:Type XI / *mecC*]	1.0E+04	
S. argenteus	**Methicillin Resistant *Staphylococcus argenteus***		
	DSM 28299 [MSHR-1132]	1.00E+05	

[a] *mec*A/C and MREJ assays positive in less than three replicates at 1.0E+04 copies/mL, no sequence based limitation on reactivity identified.

[b] Bacteria obtained through NARSA for distribution by BEI Resources, NIAID, NIH: *Staphylococcus aureus*, Strain HIP12899, NR-46081

[c] See Table 63 for limitation.

[d] MREJ type xix characterized as MSSA.[133]

Table 91. *In Silico* Reactivity Predictions for *mecA/C* and MREJ (MRSA)

mecA/C[a,b]		MREJ[d]		
Detected	**Reduced Reactivity or Not Detected**	**Detected**	**Reduced Reactivity or Not Detected**	**Unknown Reactivity (no sequences)**
mecA in *S. aureus*[c]	*mecA* in some isolates of *S. capitis*, *S. kloosii* and *S. vitulinus*	MREJ i, iα – vii[e]	MREJ ix[f]	MREJ viii
		MREJ xi-xiv	MREJ xv[g]	MREJ x
mecC in *S. aureus*		MREJ xvi – xvii	MREJ xviii	
mecA and *mecC* in non-aureus staphylococci (including *S. argenteus*)	*mecC* in *S. sciuri*	MREJ in *S. argenteus*	MREJ xix, xx[h]	
			MREJ in non-aureus staphylococci and other species[d]	

[a] July 2016; analysis of 1,257 database *mecA* sequences from *S. aureus* and 14 *mecC* sequences from *S. aureus*, as well as *mecA* and *mecC* sequences from non-aureus staphylococci.

[b] *mecC* is also referenced as SCC*mec* XI and *mecA*$_{LGA251}$.

[c] Limited or reduced reactivity predicted for 2/1,257 *mecA* sequences from *S. aureus* (0.2%).

[d] June 2016; analysis of approximately 1,450 typed MREJ database sequences from *S. aureus* and untyped sequences from *S. aureus*, non-aureus staphylococci and non-staphylococcus species (*Bacillus cereus*, *bacillus thuringiensis*, *Macrococcus caseolyticus*, *Clostridium acidurici*, *and Rummeliibacillus stabekisii*).

[e] Limited or reduced reactivity predicted for 1/141 MREJ iii sequences (0.7%); normal reactivity observed for the isolate of MREJ iii tested (see Table 90).

[f] Limited or reduced reactivity predicted for 2/8 MREJ ix sequences (25.0%); normal reactivity observed for the isolate of MREJ ix tested (see Table 90).

[g] Reduced reactivity predicted by *in silico* analysis (for approximately 40% of MREJ xv-like sequences evaluated in March 2022) and observed with the isolate of MREJ xv tested (see Table 90 and Table 63) .

[h] MREJ xix and xx were not included in the assay design because they were identified from methicillin-sensitive *S. aureus.*[133]

Table 92. Isolates Containing the *bla*$_{CTX-M}$ gene Tested and Detected, and *In Silico* Reactivity Predictions

CTX-M Type	Organism	Source	Test concentration (copies/mL)	Result
Isolates Tested				
CTX-M	*E. coli*	AR-Bank #0137[a]	1.0E+04	CTX-M Detected
	K. oxytoca	GRE 1254054	1.0E+04	
	K. pneumoniae	AR-Bank #0068[a]	1.0E+04	
		AR-Bank #0153[a]	1.0E+04	
		GRE 1355030	1.0E+04	
CTX-M-1	*E. coli*	AR-Bank #0162	1.0E+04	
CTX-M-2	*K. pneumoniae*	AR-Bank #0107	1.0E+04	
CTX-M-8	*K. aerogenes*	GRE 1254066	1.0E+04	
CTX-M-9	*E. coli*	AR-Bank #0086	1.0E+04	
	E. cloacae	NCTC 13464	1.0E+04	
CTX-M-14	*K. pneumoniae*	AR-Bank #0079	1.0E+04	
CTX-M-15	*E. coli*	Zeptometrix 0801905	1.0E+04	
CTX-M-22	*P. mirabilis*	GRE 1254053	1.0E+04	
CTX-M-25	*K. pneumoniae*	NCTC 13465	1.0E+04	

***In silico* Reactivity Predictions[a]**				
Detected		**Not Detected**	**Unknown Reactivity (no sequences)**	
CTX-M-1 – CTX-M-117	CTX-M-150	CTX-M-151	CTX-M-118	CTX-M-143
CTX-M-121 – CTX-M-126	CTX-M-152		CTX-M-119	CTX-M-145
CTX-M-129 – CTX-M-132	CTX-M-155 – CTX-M-177		CTX-M-120	CTX-M-146
CTX-M-134	CTX-M-179 – CTX-M-185		CTX-M-127	CTX-M-149
CTX-M-136 – CTX-M-139			CTX-M-128	CTX-M-153
CTX-M-141 – CTX-M-142			CTX-M-133	CTX-M-154
CTX-M-144			CTX-M-135	CTX-M-178
CTX-M-147 – CTX-M-148			CTX-M-140	

[a] July 2016; analysis of over 1,200 database CTX-M sequences (typed and untyped).

Table 93. Isolates Containing the *bla*$_{IMP}$ gene Tested and Detected, and *In Silico* Reactivity Predictions

IMP Type	Organism	Source	Test concentration (copies/mL)	Result
Isolates Tested				
IMP	*K. aerogenes*	AR-Bank #0161	1.0E+04	IMP Detected
	E. coli	GRE 1062016	1.0E+04	
	K. pneumoniae	AR-Bank #0080	1.0E+04	
IMP-1[a]	*P. aeruginosa*	AR-Bank #0103	1.0E+04	
IMP-3[a]	*E. coli*	GRE 1252008	1.0E+04	
IMP-4	*A. baumannii*	GRE 1062081	1.0E+04	
IMP-8	*K. pneumoniae*	GRE 1062084	1.0E+04	
IMP-9	*E. coli*	GRE 1252009	1.0E+04	
IMP-14	*P. aeruginosa*	AR-Bank #0092	1.0E+04	

***In silico* Reactivity Predictions[b]**				
Detected			**Reduced Reactivity or Not Detected**	**Unknown Reactivity (no sequences)**
IMP-1 – IMP-30[a]	IMP-40 – IMP-45	IMP-58 – IMP- 60	IMP-31	IMP-36
			IMP-35	IMP-39
IMP-32 – IMP-34	IMP-48 – IMP-49			IMP-46
				IMP-47
IMP 37 – IMP-38	IMP-51 – IMP-56			IMP-50
				IMP-57

[a] Limited or reduced reactivity predicted for 1/36 (2.8%) of IMP-1 and 1/3 (33.3%) of IMP-3 sequences.

[b] June 2016; analysis of over 220 database IMP sequences (typed and untyped).

Table 94. Isolates Containing the *bla*$_{KPC}$ gene Tested and Detected, and *In Silico* Reactivity Predictions

KPC Type	Organism	Source	Test concentration (copies/mL)	Result
Isolates Tested				

KPC	*E. cloacae*	ATCC BAA-2341	1.0E+04	KPC Detected
	E. hormaechi	ATCC BAA-2082	1.0E+04	
	P. mirabilis	AR-Bank #0156	1.0E+04	
	K. oxytoca	AR-Bank #0147	1.0E+04	
	K. pneumoniae	AR-Bank #0097	1.0E+04	
	K. oxytoca	JMI 2523	1.0E+04	
KPC-2	*K. oxytoca*	JMI 7818	1.0E+04	
	K. pneumoniae	Zeptometrix 0801886	1.0E+04	
	K. pneumoniae	JMI 328	1.0E+04	
	K. pneumoniae	ATCC BAA-1705	1.0E+04	
	S. marcescens	JMI 697	1.0E+04	
KPC-3	*E. coli*	AR-Bank #0061	1.0E+04	
	K. oxytoca	JMI 2661	1.0E+04	
KPC-4	*K. pneumoniae*	JMI 766	1.0E+04	
KPC-5	*P. aeruginosa*	Creighton University PS28	1.0E+04	
***In silico* Reactivity Predictions[a]**				
Detected			**Not Detected**	**Unknown Reactivity (no sequences)**
KPC-1-19	KPC-21-22	KPC-24-26	None	KPC-20
				KPC-23

[a] August 2016; analysis of approximately 1,125 database KPC sequences (typed and untyped).

Table 95. Isolates Containing the *bla*$_{NDM}$ gene Tested and Detected, and *In Silico* Reactivity Predictions

NDM Type	Organism	Source	Test concentration (copies/mL)	Result
Isolates Tested				
NDM	*E. coli*	AR-Bank #0162	1.0E+04	NDM Detected
	K. pneumoniae	AR-Bank #0153	1.0E+04	
	K. pneumoniae	AR-Bank #0068	1.0E+04	
	P. mirabilis	AR-Bank #0159	1.0E+04	
NDM-1[a]	*A. baumannii*	AR-Bank #0033	1.0E+04	
NDM-2	*A. baumannii*	GRE 1153064	1.0E+04	
NDM-5	*E. coli*	AR-Bank #0150	1.0E+04	
NDM-6	*E. coli*	AR-Bank #0137	1.0E+04	
***In silico* Reactivity Predictions[b]**				
Detected			**Not Detected**	
NDM-1[a]	NDM-7	NDM-13	None	
NDM-2	NDM-8	NDM-14		
NDM-3	NDM-9	NDM-15		
NDM-4	NDM-10	NDM-16		
NDM-5	NDM-11			
NDM-6	NDM-12			

[a] Limited or reduced reactivity is predicted for 3/430 NDM-1 sequences (0.7%).

[b] June 2016; analysis of 900 database NDM sequences (typed and untyped).

Table 96. Isolates Containing the *bla*$_{OXA-48}$ and like genes Tested and Detected, and *In Silico* Reactivity Predictions

OXA-48-like Type[a]	Organism	Source	Test concentration (copies/mL)	Result
Isolates Tested and Detected				
OXA-48	*K. aerogenes*	AR-Bank #0074	1.0E+04	OXA-48-like Detected
OXA-48-like	*S. marcescens*	GRE 1411136	1.0E+04	
	S. marcescens	GRE 1411137	1.0E+04	
OXA-162	*K. pneumoniae*	GRE 1355030	1.0E+04	
OXA-181	*K. pneumoniae*	AR-Bank #0068	1.0E+04	
OXA-232	*K. pneumoniae*	AR-Bank #0075	1.0E+04	
***In silico* Reactivity Predictions[a]**				
Detected			**Not Detected[b]**	
OXA-48	OXA-204	OXA-370	OXA-163[c]	OXA-438[c]
OXA-48-like	OXA-232	OXA-484	OXA-247[c]	OXA-439[c]
OXA-162	OXA-244	OXA-505	OXA-405[c]	
OXA-181	OXA-245		OXA-416	
OXA-199	OXA-252		OXA-436[c]	

[a] June 2016; analysis of 165 database OXA-48-like sequences (typed and untyped).

[b] Sequence analysis predicts that the listed OXA-48-like types will not be detected. Non-OXA-48-like types (e.g. OXA-23-like, OXA-40/24-like, OXA-51-like, and OXA-58-like, OXA-143a-like, and OXA-143-like) will also not be detected.

[c] Deletion variants with altered carbapenem hydrolysis activity, as described for OXA-163.[134]

Table 97. Isolates Containing the *bla*$_{VIM}$ gene Tested and Detected, and *In Silico* Reactivity Predictions

VIM Type	Organism	Source	Test concentration (copies/mL)	Result
Isolates Tested				
VIM	*E. cloacae*	AR-Bank #0154	1.0E+04	VIM Detected
	P. aeruginosa	AR-Bank #0111	1.0E+04	
	K. pneumoniae	AR-Bank 0076	1.0E+04	
VIM-2[a]	*P. aeruginosa*	AR-Bank #0100	1.0E+04	
VIM-4	*P. aeruginosa*	AR-Bank #0054	1.0E+04	
VIM-7	*E. coli*	GRE 1256018	1.0E+04	
VIM-10	*P. aeruginosa*	NCTC 13437	1.0E+04	
***In silico* Reactivity Predictions[b]**				
Detected			**Reduced Reactivity or Not Detected**	**Unknown Reactivity (no sequences)**
VIM-1 – VIM-20[a]	VIM-23 – VIM-47	VIM-49 – VIM-51	VIM-39	VIM-21
			VIM-45	VIM-22
			VIM-46	VIM-48

[a] Limited or reduced reactivity is predicted for 2/177 VIM-2 sequences (1.1%).

[b] September 2016; analysis of over 600 database VIM sequences (typed and untyped).

Analytical Specificity (Cross-Reactivity and Exclusivity)

There is a risk of false positive results due to non-specific amplification and/or cross-reactivity with organisms found in the respiratory tract. The potential for non-specific amplification and detection by the BIOFIRE Pneumonia Panel assays was evaluated by *in silico* analyses of available sequences and by empirical (wet) testing of high concentrations of organisms in contrived samples. The observed and predicted cross-reactivities for organisms closely related to those detected by the panel and unrelated organisms that may be present in lower respiratory specimens are summarized in Table 98. Erroneous results due to cross-reactivity with organisms that were not evaluated or new variant sequences that emerge is also possible.

On-panel organisms were tested to assess the potential for intra-panel cross-reactivity (Table 99). Off-panel organisms included species of the same genus or otherwise genetically related to organisms detected by the panel, as well as normal flora and pathogens that may be present in sputum-like and BAL-like specimens (Table 100). Antimicrobial resistance genes were also evaluated in conjunction with on and off panel host organisms.

The final concentration of analyte in the sample (typically ≥1.0E+07 CFU/mL for bacteria and fungi and ≥1.0E+05 $TCID_{50}$/mL for viruses) represented levels ~100 - 100,000 fold higher than the LoD or lowest reportable level of the BIOFIRE Pneumonia Panel assays.

Table 98. Observed and Predicted Cross-Reactivity of BIOFIRE Pneumonia Panel Assays

BIOFIRE Pneumonia Panel Result	Cross-Reactive Organism
Closely-Related Species	
Enterobacter cloacae complex	*Enterobacter bugandensis*[a], *E. chengduensis*[a]
Escherichia coli	*Escherichia fergusonii*[b]
	Shigella species *(S. boydii, S. dysenteriae, S. flexneri, S. sonnei)*[b]
Klebsiella oxytoca	*Klebsiella michiganensis*[b]
Staphylococcus aureus	*Staphylococcus argenteus*[c]
	Staphylococcus schweitzeri[d]
Pseudomonas aeruginosa	*Pseudomonas putida*[e]
Unrelated Species	
Human rhinovirus/enterovirus	*Bordetella* species[f]
Parainfluenza virus[g]	*Aspergillus niger*
	Cryptococcus laurentii
	Cryptococcus uniguttulatus
Adenovirus	*Stenotrophomonas acidaminiphila*[h]
CTX-M[i]	*Acinetobacter schindleri*
	Burkholderia vietnamiensis[j]
Escherichia coli[k,l]	*Lelliottia amnigena (Enterobacter amnigenus)*
	Enterobacter cloacae complex species[l]

[a] Not tested, predicted by *in silico* analysis only. The designation of some *Enterobacter* species (e.g. *E. bugandensis*, *E. cancerogenus, E. chengduensis*, etc.) as members of the *Enterobacter cloacae* complex is uncertain. *In silico* analysis predicts that the assay will detect (cross-react with) *E. chengduensis* and a subset (~30%) of the E. bugandensis sequences evaluated. *In silico* analysis and testing indicates the assay will not detect (cross-react with) *E. cancerogenus*.

[b] Genetically or phenotypically indistinguishable and often misidentified by standard laboratory techniques. Detected at a concentrations ≥1.0E+04 copies/mL.

[c] Genetically or phenotypically indistinguishable and often misidentified by standard laboratory techniques. Detected at a concentrations ≥1.0E+05 copies/mL.

[d] Genetically or phenotypically indistinguishable and often misidentified by standard laboratory techniques. Detected at a concentrations ≥1.0E+06 copies/mL.

[e] Cross-reactivity possible at concentrations >1.0E+07 copies/mL.

[f] Cross-reactivity with *B. pertussis* confirmed at ≥1.0E+06 CFU/mL. Cross-reactivity with *B. parapertussis* and *B. bronchiseptica* was not observed at 1.0E+08 CFU/mL, but possible based on sequence analysis.

[g] Cross-reactivity was observed with *A. niger*, *C. laurentii,* and *C. uniguttulatus* at concentrations >1.0E+06 copies/mL. Cross-reactivity with other *Cryptococcus* species may be possible based on sequence analysis.

[h] *S. acidaminiphila* has not been isolated from human clinical specimens, no cross-reactivity observed with other *Stenotrophomonas* species.

[i] Cross-reactive product observed only at concentrations >4.5E+07 CFU/mL and only reported if an applicable gram-negative bacterium is also detected.

[j] Not tested. Predicted by *in silico* analysis.

[k] If observed, results will be reported as *Escherichia coli* 10^4 copies/mL.

[l] Based on *in silico* analysis, the observed cross-reactivity at high concentrations (≥1.0E+08 copies/mL) is predicted only for a subset of sequences from various *Enterobacter cloacae* complex species (*E. asburiae*, *E. cloacae*, *E. hormaechei* and *E. ludwigii*) and similar weak cross-reactivity is also possible with *Escherichia albertii* and select strains/sequences of *Klebsiella* (*Enterobacter*) *aerogenes* (not observed when tested).

Table 99. On Panel Organisms Tested for Evaluation of BIOFIRE Pneumonia Panel Analytical Specificity

False positive results were observed when testing the species shown in **bold**.

ON-PANEL			
Bacteria			
Acinetobacter baumannii	***Enterobacter ludwigii***[a]	*Klebsiella variicola*	*Serratia marcescens*
Acinetobacter calcoaceticus	*Escherichia coli*	*Moraxella catarrhalis*	*Staphylococcus aureus*
Acinetobacter nosocomialis	*Haemophilus influenzae*	*Proteus hauseri*	*Streptococcus agalactiae*
Acinetobacter pittii	*Klebsiella aerogenes*	*Proteus mirabilis*	*Streptococcus pneumoniae*
Enterobacter asburiae[a]	*Klebsiella oxytoca*	*Proteus penneri*	*Streptococcus pyogenes*
Enterobacter cloacae[a]	*Klebsiella pneumoniae*	*Proteus vulgaris*	
Enterobacter hormaechei[a]	*Klebsiella quasipneumoniae*	*Pseudomonas aeruginosa*	
Atypical Bacteria			
Chlamydia pneumoniae	*Legionella pneumophila*	*Mycoplasma pneumoniae*	
Viruses			
Adenovirus B	Coronavirus NL63	Human metapneumovirus	Parainfluenza virus 2
Adenovirus C	Coronavirus OC43	Influenza A virus	Parainfluenza virus 3
Adenovirus E	Enterovirus	Influenza B virus	Parainfluenza virus 4
Coronavirus 229E	Human rhinovirus	Parainfluenza virus 1	Respiratory syncytial virus
Coronavirus HKU1			
Antimicrobial Resistance Genes			
CTX-M (*Klebsiella oxytoca*)	KPC (*Klebsiella pneumoniae*)	OXA-48-like (*Serratia marcescens*)	*mecA* and MREJ (MRSA) (*Staphylococcus aureus*)
IMP (*Pseudomonas aeruginosa*)	NDM (*Acinetobacter baumannii*)	VIM (*Enterobacter cloacae*)	

[a] See Table 98 for cross-reactivity information.

Table 100. Off-Panel Bacteria Tested or Evaluated by In Silico Analysis for BIOFIRE Pneumonia Panel Analytical Specificity

False positive results were observed when testing the species shown in **bold**.

OFF-PANEL			
Bacteria			
Abiotrophia defectiva	***Escherichia fergusonii***[a]	*Mycobacterium tuberculosis*	***Shigella boydii***[a]
Achromobacter xylosoxidans	*Escherichia hermanii*	*Mycoplasma bovis*	***Shigella dysenteriae***[a]
Acinetobacter haemolyticus	*Escherichia vulneris*	*Mycoplasma genitalium*	***Shigella flexneri***[a]
Acinetobacter johnsonii	*Fluoribacter dumoffei*	*Mycoplasma hominis*	***Shigella sonnei***[a]
Acinetobacter junii	*Fusobacterium varium*	*Mycoplasma orale*	***Staphylococcus argenteus***[a]
Acinetobacter lwolfii	*Gemella morbillorum*	*Neisseria gonorrhoeae*	*Staphylococcus capitis*
Acinetobacter radioresistens	*Granulicatella adiacens*	*Neisseria lactamica*	*Staphylococcus caprae*
Acinetobacter schindleri[a]	*Haemophilus ducreyi*	*Neisseria meningitidis*	*Staphylococcus cohnii*
Acinetobacter ursingii	*Haemophilus haemolyticus*	*Neisseria mucosa*	*Staphylococcus haemolyticus*
Actinobacillus actinomycetemcomitans	*Haemophilus parahaemolyticus*	*Neisseria sicca*	*Staphylococcus epidermidis* (*mecA*)
Actinobacillus hominis	*Haemophilus parainfluenzae*	*Nocardia asteroides*	*Staphylococcus hominis*
Actinobacillus ureae	*Haemophilus parasuis*	*Nocardia brasilensis*	*Staphylococcus intermedius*
Actinomyces israelii	*Haemophilus sputorum*[b]	*Pantoea agglomerans*	*Staphylococcus lugdunensis*
Actinomyces naeslundii	*Hafnia alvei*	*Pasteurella multocida*	*Staphylococcus lutrae*
Bacillus cereus	*Hafnia paralvei*	*Pediococcus acidilactici*	*Staphylococcus pasteuri*

OFF-PANEL			
Bacteriodes fragilis	*Helicobacter pylori*	*Peptostreptococcus anaerobius*	*Staphylococcus pseudointermedius*
Bordatella bronchiseptica	*Kingella kingae*	*Pluralibacter gergoviae*	*Staphylococcus saprophyticus*
Bordatella parapertussis	***Klebsiella michiganensis***[a]	*Porphyromonas gingivalis*	*Staphylococcus schleiferi*
Bordatella pertussis[a]	*Kluyvera intermedia*	*Prevotella intermedia*	***Staphylococcus schweitzeri***[a]
Burkholderia cepacia	*Kluyvera ascorbata*	*Prevotella melaninogenica*	*Staphylococcus sciuri*
Burkholderia mallei	*Lactobacillus acidophilus*	*Prevotella oralis*	*Staphylococcus warneri*
Burkholderia multivorans	*Leclercia adecarboxylata*	*Propionibacterium acnes*	*Staphylococcus xylosus*
Burkholderia pseudomallei	*Legionella bozemanii*	*Providencia rettgeri* (OXA-48-like)	***Stenotrophomonas acidaminiphila***[a]
Cardiobacterium hominis	*Legionella cincinnatiensis*	*Providencia stuartii*	*Stenotrophomonas maltophilia*
Cedecea davisae	*Legionella feeleii*	*Pseudomonas fluorescens*	*Stenotrophomonas nitritireducens*
Chlamydia trachomatis	*Legionella lansingensis*	*Pseudomonas luteola*	*Stenotrophomonas rhizophila*
Chlamydophila psittaci	*Legionella longbeachae*	*Pseudomonas nitroreducens*	*Streptococcus equi* subsp. *zooepidemicus*
Citrobacter freundii (KPC)	*Legionella micdadei*	*Pseudomonas oryzihabitans*	*Streptococcus mitis*
Citrobacter koseri (OXA-48-like)	*Legionella wadsworthii*	*Pseudomonas pertucinogena*	*Streptococcus mutans*
Citrobacter sedlakii	*Lelliottia nimipressuralis*	***Pseudomonas putida***[a] (IMP)	*Streptococcus oralis*
Citrobacter werkmanii (VIM)	***Lelliottia amnigena***[a] *(Enterobacter amnigenus)*	*Pseudomonas stutzeri*	*Streptococcus parasanguinis*
Clostridium difficile	*Leuconostoc lactis*	*Ralstonia pickettii*	*Streptococcus pseudopneumoniae*
Clostridium perfringens	*Listeria monocytogenes*	*Raoultella ornithinolytica*	*Streptococcus salivarius*
Corynebacterium diptheriae	*Macrococcus caseolyticus*	*Raoultella planticola*	*Streptococcus sanguinis*
Corynebacterium genitalium	*Micrococcus luteus*	*Raoultella terrigena*	*Streptococcus tigurinus*
Corynebacterium pseudodiptherticum	*Moraxella equi*	*Rhodococcus equi*	*Streptomyces anulatus*[c]
Corynebacterium urealyticum	*Moraxella lacunata*	*Rothia mucilaginosa*	*Treponema denticola*
Cronobacter sakazakii	*Moraxella lincolnii*	*Salmonella enterica* (CTX-M)	*Ureaplasma parvum*
Eikenella corrodens	*Moraxella nonliquiefaciens*	*Serratia fonticola*	*Ureaplasma urealyticum*
Enterobacter cancerogenus	*Morganella morganii* (NDM)	*Serratia liquefaciens*	*Vagococcus fluvialis*
Enterobacter massiliensis	*Mycobacterium africanum*[b]	*Serratia odorifera*	*Veillonella parvula*
Enterobacter soli	*Mycobacterium bovis*	*Serratia plymuthica*	*Yersinia enterocolitica*
Enterococcus faecium	*Mycobacterium caprae*[c]	*Serratia rubidaea*	*Yersinia pseudotuberculosis*
Enterococcus faecalis	*Mycobacterium microti*[b]		
Viruses			
Bocavirus	Hantavirus[b]	Human papillomavirus (HPV)	Mumps virus
Cytomegalovirus	Herpes simplex virus 1	Influenza C virus[b]	Varicella zoster virus
Epstein Barr virus	Human immunodeficiency virus (HIV)	Middle East respiratory syndrome coronavirus (MERS-CoV)	Severe acute respiratory syndrome coronavirus (SARS-CoV)
German measles virus (Rubella)	Measles Virus (Rubeola)		
Fungi/Yeast			
Aspergillus flavus	*Coccidioides posadasii*	*Fusarium kyushense*	*Pneumocystis carinii*
Aspergillus fumigatus	*Cryptococcus albidus*	*Histoplasma capsulatum*[c]	*Pneumocystis jirovecii*
Aspergillus niger[a]	*Cryptococcus gattii*	*Paecilomyces variottii*	*Pneumocystis murina*
Aspergillus terreus	***Cryptococcus laurentii***[a]	*Paracoccidodes brasiliensis*[b]	*Rhizopus microsporus*
Blastomyces dermatitidis	*Cryptococcus neoformans*	*Penicillium chrysogenum*[c]	*Scedosporium apiospermum*
Candida albicans	***Cryptococcus uniguttalatus***[a]	*Penicillium marneffei*	*Scedosporium prolificans*
Candida glabrata	*Filobasidium capsuligenum*		
Antimicrobial Resistance Genes			
AmpC (Klebsiella (*Enterobacter) aerogenes*)	OXA-24/40 (non-48-like) (*Acinetobacter baumannii*)	SME (*Serratia marcescens*)	TEM (*Escherichia coli*)
CMY (II) (*Escherichia coli*)	SHV (*Klebsiella pneumoniae*)	SPM (*Pseudomonas aeruginosa*)	VAN (*Staphylococcus aureus*)
ompK36 [SHV-12,OMPC] (*Klebsiella pneumoniae*)	*SCCmec* variant lacking *mecA* or *mecC* (*Staphylococcus aureus*)[d]		

[a] See Table 98 for cross-reactivity information.

[b] Analytical specificity was evaluated only by in silico analysis of whole genome or partial genome sequences in public databases. No cross-reactivity is predicted based on the sequences analyzed.

[c] Tested at a concentration less than 1.0E+07 CFU/mL and also evaluated by in silico analysis. Cross-reactivity was not observed in testing nor predicted based on the sequences analyzed.

[d] Methicillin-sensitive isolate of *S. aureus* (Rennes 1060728) that has MREJ sequence but no *mecA* or *mecC* gene (empty cassette). *Staphylococcus aureus* was reported as Detected and the *mecA/C* and MREJ (MRSA) result was Not Detected.

Precision (Reproducibility)

Precision (Reproducibility) testing was performed with contrived BAL samples over multiple days at three laboratory locations (sites) on a combination of BIOFIRE systems. The testing incorporated a range of potential variation introduced by operator, system, instrument or module, concentration and reagent lot, for a total of 30 tests per system and 90 total replicates per sample/concentration.

Evaluation of the reproducibility of Detected/Not Detected results for atypical bacteria and viruses included samples containing combinations of five different analytes, at Negative, Low Positive (1×LoD), and Moderate Positive (3×LoD) concentrations. Negative results were obtained from samples that were not spiked with the analyte (see evaluation of precision for bacterial analytes below).

A summary of results (percent (%) agreement with the expected Detected or Not Detected result) for atypical bacteria and viruses (by site and system) is provided in Table 101.

Table 101. Reproducibility of BIOFIRE Pneumonia Panel Atypical Bacteria and Virus Results

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result			
			BIOFIRE	BIOFIRE 2.0	BIOFIRE TORCH	All Sites/Systems [95% CI]
			Site A	Site B	Site C	
Atypical Bacteria						
Chlamydia pneumoniae	**None** (No Analyte)	**Not Detected**	**780/780** 100%	**780/780** 100%	**780/780** 100%	**2,340/2,340** **100%** [99.8%-100%]
Legionella pneumophila Philadelphia-1 ATCC 33152	**Moderate Positive** **3× LoD** 1.5E+03 CFU/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** [96.0%-100%]
	Low Positive **1× LoD** 5.0E+02 CFU/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** [96.0%-100%]
	None (No Analyte)	**Not Detected**	**720/720** 100%	**720/720** 100%	**720/720** 100%	**2,160/2,160** **100%** [99.8%-100%]
Mycoplasma pneumoniae	**None** (No Analyte)	**Not Detected**	**780/780** 100%	**780/780** 100%	**780/780** 100%	**2,340/2,340** **100%** [99.8%-100%]
Viruses						
Adenovirus Species B Serotype 3 ZeptoMetrix 0810062CF	**Moderate Positive** **3× LoD** 3.0E+00 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** [96.0%-100%]
	Low Positive **1× LoD** 1.0E+00 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** [96.0%-100%]
	None (No Analyte)	**Not Detected**	**720/720** 100%	**720/720** 100%	**720/720** 100%	**2,160/2,160** **100%** [99.8%-100%]
Coronavirus	**None** (No Analyte)	**Not Detected**	**780/780** 100%	**776/780** 99.5%	**780/780** 100%	**2,336/2,340** **99.8%** [99.6%-100%]
Human metapneumovirus 16 Type A1 ZeptoMetrix 0810161CF	**Moderate Positive** **3× LoD** 1.5E+02 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** [96.0%-100%]
	Low Positive **1× LoD** 5.0E+01 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**29/30** 96.7%	**30/30** 100%	**89/90** **98.9%** [94.0%-100%]
	None (No Analyte)	**Not Detected**	**720/720** 100%	**720/720** 100%	**720/720** 100%	**2,160/2,160** **100%** [99.8%-100%]
Human rhinovirus/enterovirus	**None** (No Analyte)	**Not Detected**	**779/780** 99.9%	**780/780** 100%	**779/780** 99.9%	**2,338/2,340** **99.9%** [99.7%-100%]

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result			
			BIOFIRE	BIOFIRE 2.0	BIOFIRE TORCH	All Sites/Systems [95% CI]
			Site A	Site B	Site C	
Influenza A virus H3N2 A/Port Chalmers/1/73 ATCC VR-810	**Moderate Positive 3× LoD** 1.5E+00 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** [96.0%-100%]
	Low Positive 1× LoD 0.5E-01 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**29/30** 96.7%	**30/30** 100%	**89/90 98.9%** [94.0%-100%]
	None (No Analyte)	**Not Detected**	**720/720** 100%	**720/720** 100%	**720/720** 100%	**2,160/2,160 100%** [99.8%-100%]
Influenza B virus	**None** (No Analyte)	**Not Detected**	**780/780** 100%	**780/780** 100%	**780/780** 100%	**2,340/2,340 100%** [99.8%-100%]
Parainfluenza virus Type 2 ZeptoMetrix 0810015CF	**Moderate Positive 3× LoD** 7.5E+01 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** [96.0%-100%]
	Low Positive 1× LoD 2.5E+01 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** [96.0%-100%]
	None (No Analyte)	**Not Detected**	**720/720** 100%	**720/720** 100%	**720/720** 100%	**2,160/2,160 100%** [99.8%-100%]
Respiratory syncytial virus	**None** (No Analyte)	**Not Detected**	**780/780** 100%	**780/780** 100%	**780/780** 100%	**2,340/2,340 100%** [99.8%-100%]

Precision for bacterial analytes was measured at each concentration as 1) precision of bin results and 2) reproducibility of analyte detection. When a sample containing one or more bacteria is tested repeatedly, the precision of the bin results (probability that each replicate will receive the same bin result) will vary based on the concentration of nucleic acid measured and the relation of that concentration to the limits of each bin. Bin precision may be as low as 50% for values at a bin limit and precision will increase (up to 90% or higher) as the distance of the measured value from a bin limit increases. The precision of BIOFIRE Pneumonia Panel bin results will follow the model illustrated in Figure 2 :

- >90% at a bin center (Scenario 1),
- ~60 – 90% between a bin limit and bin center (Scenario 2), and
- ~50% at bin limits (Scenario 3).

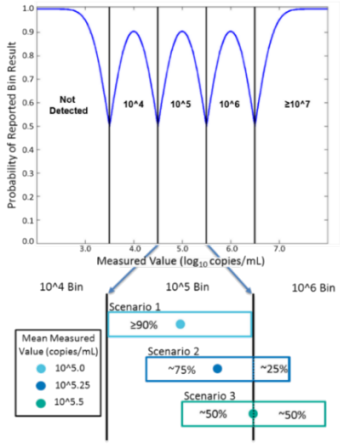


Figure 2. Model for Precision of BIOFIRE Pneumonia Panel Bin Results
Top: The probability (0.0 – 1.0) of the same bin results for each replicate tested varies based on proximity of the measured value to a bin limit.
Bottom: Expected distribution of bin results at different mean measured values.

Samples containing bacteria and corresponding antimicrobial resistance (AMR) genes were tested at six different concentrations over the reportable range and below. A summary of the bin precision (percent (%) of replicates reported in each bin) and the reproducibility of detection is shown at each concentration tested in Table 102.

Table 102. Reproducibility of BIOFIRE Pneumonia Panel Bacterial Bin Results

Grey shading indicates the expected bin results based on the analyte concentration and bold font indicates the bin with the greatest percentage of results at each concentration.

Analyte	Concentration (log_{10} copies/mL)	% Replicates Reported in Each Bin Result					Total Detected
		≥10^7	10^6	10^5	10^4	ND	
Acinetobacter baumannii (NDM-1) AR-BANK#0033	**7.5**	**90/90 (100%)**	-	-	-	-	**90/90** 100%
	6.5	**87/90 (96.7%)**	3/90 (3.3%)	-	-	-	**90/90** 100%
	5.5	-	**82/90 (91.1%)**	8/90 (8.9%)	-	-	**90/90** 100%
	4.5	-	1/90 (1.1%)	**80/90 (88.9%)**	9/90 (10.0%)	-	**90/90** 100%
	3.5	-	-	1/90 (1.1%)	**74/90 (82.2%)**	15/90 (16.7%)	**75/90** 83.3%
	2.5	-	-	-	1/90 (1.1%)	**89/90 (98.9%)**	**1/90** 1.1%
	None (No Analyte)	-	-	-	-	**1800/1800 (100%)**	**0/1800** 0.0%
Enterobacter cloacae (VIM) AR-BANK#0154	**7.0**	**90/90 (100%)**	-	-	-	-	**90/90** 100%
	6.0	4/90 (4.4%)	**86/90 (95.6%)**	-	-	-	**90/90** 100%
	5.0	-	6/90 (6.7%)	**80/90 (88.9%)**	-	4/90 (4.4%)	**86/90** 95.6%
	4.0	-	-	6/90 (6.7%)	**83/90 (92.2%)**	1/90 (1.1%)	**89/90** 98.9%
	3.0	-	-	1/90 (1.1%)	4/90 (4.4%)	**85/90 (94.4%)**	**5/90** 5.6%
	2.0	-	-	-	-	**90/90 (100%)**	**0/90** 0.0%
	None (No Analyte)	-	-	-	-	**1800/1800 (100%)**	**0/1800** 0.0%
Escherichia coli (IMP) GRE 1062016	**7.0**	**90/90 (100%)**	-	-	-	-	**90/90** 100%
	6.0	7/90 (7.8%)	**82/90 (91.1%)**	-	-	1/90 (1.1%)	**89/90** 98.9%
	5.0	-	10/90 (11.1%)	**80/90 (88.9%)**	-	-	**90/90** 100%
	4.0	-	-	12/90 (13.3%)	**77/90 (85.6%)**	1/90 (1.1%)	**89/90** 98.9%
	3.0	-	-	1/90 (1.1%)	15/90 (16.7%)	**74/90 (82.2%)**	**16/90** 17.8%
	2.0	-	-	-	-	**90/90 (100%)**	**0/90** 0.0%
	None (No Analyte)	-	-	-	-	**1800/1800 (100%)**	**0/1800** 0.0%
Haemophilus influenzae ATCC 10211	**7.0**	**89/90 (98.9%)**	1/90 (1.1%)	-	-	-	**90/90** 100%
	6.0	35/90 (48.9%)	**55/90 (61.1%)**	-	-	-	**90/90** 100%
	5.0	-	**49/90 (54.4%)**	40/90 (44.4%)	1/90 (1.1%)	-	**90/90** 100%
	4.0	-	-	41/90 (45.6%)	**49/90 (54.4%)**	-	**90/90** 100%
	3.0	-	-	-	42/90 (46.7%)	**48/90 (53.3%)**	**42/90** 46.7%
	2.0	-	-	-	-	**90/90 (100%)**	**0/90** 0.0%
	None (No Analyte)	-	-	-	-	**1800/1800 (100%)**	**0/1800** 0.0%
Klebsiella aerogenes (Enterobacter	**7.5**	**90/90 (100%)**	-	-	-	-	**90/90** 100%

Analyte	Concentration (log_{10} copies/mL)	% Replicates Reported in Each Bin Result					Total Detected
		≥10^7	10^6	10^5	10^4	ND	
aerogenes) ATCC 13048	**6.5**	**65/90 (72.2%)**	25/90 (27.8%)	-	-	-	**90/90** 100%
	5.5	-	**52/90 (57.8%)**	38/90 (42.2%)	-	-	**90/90** 100%
	4.5	-	-	38/90 (42.2%)	**51/90 (56.7%)**	1/90 (1.1%)	**89/90** 98.9%
	3.5	-	-	-	33/90 (36.7%)	**57/90 (63.3%)**	**33/90** 36.7%
	2.5	-	-	-	1/90 (1.1%)	**89/90 (98.9%)**	**1/90** 1.1%
	None (No Analyte)	-	-	-	-	**1800/1800 (100%)**	**0/1800** 0.0%
Klebsiella oxytoca (CTX-M) GRE 1254054	**7.5**	**90/90 (100%)**	-	-	-	-	**90/90** 100%
	6.5	**90/90 (100%)**	-	-	-	-	**90/90** 100%
	5.5	1/90 (1.1%)	**84/90 (93.3%)**	3/90 (3.3%)	-	2/90 (2.2%)	**88/90** 97.8%
	4.5	-	-	**89/90 (98.9%)**	-	1/90 (1.1%)	**89/90** 98.9%
	3.5	-	-	-	**90/90 (100%)**	-	**90/90** 100%
	2.5	-	-	1/90 (1.1%)	1/90 (1.1%)	**88/90 (97.8%)**	**2/90** 2.2%
	None (No Analyte)	-	-	-	-	**1800/1800 (100%)**	**0/1800** 0.0%
Klebsiella pneumoniae (KPC) AR-BANK#0097	**7.0**	**90/90 (100%)**	-	-	-	-	**90/90** 100%
	6.0	12/90 (13.3%)	**78/90 (86.7%)**	-	-	-	**90/90** 100%
	5.0	-	15/90 (16.7%)	**75/90 (83.3%)**	-	-	**90/90** 100%
	4.0	-	-	23/90 (25.6%)	**66/90 (73.3%)**	1/90 (1.1%)	**89/90** 98.9%
	3.0	-	-	-	15/90 (16.7%)	**75/90 (83.3%)**	**15/90** 16.7%
	2.0	-	-	-	-	**90/90 (100%)**	**0/90** 0.0%
	None (No Analyte)	-	-	-	-	**1800/1800 (100%)**	**0/1800** 0.0%
Moraxella catarrhalis ATCC 8176	**7.0**	**90/90 (100%)**	-	-	-	-	**90/90** 100%
	6.0	26/90 (28.9%)	**64/90 (71.1%)**	-	-	-	**90/90** 100%
	5.0	-	6/90 (6.7%)	**83/90 (92.2%)**	1/90 (1.1%)	-	**90/90** 100%
	4.0	-	-	4/90 (4.4%)	**86/90 (95.6%)**	-	**90/90** 100%
	3.0	-	-	-	4/90 (4.4%)	**86/90 (95.6%)**	**4/90** 4.4%
	2.0	-	-	-	-	**90/90 (100%)**	**0/90** 0.0%
	None (No Analyte)	-	-	-	-	**1800/1800 (100%)**	**0/1800** 0.0%
Proteus mirabilis ATCC 35659	**7.0**	**88/90 (97.8%)**	-	-	-	2/90 (2.2%)	**88/90** 97.8%
	6.0	27/90 (30.0%)	**63/90 (70.0%)**	-	-	-	**90/90** 100%
	5.0	-	26/90 (28.9%)	**64/90 (71.1%)**	-	-	**90/90** 100%
	4.0	-	-	14/90 (15.6%)	**75/90 (83.3%)**	1/90 (1.1%)	**89/90** 98.9%
	3.0	-	-	-	28/90 (31.1%)	**62/90 (68.9%)**	**28/90** 31.1%
	2.0	-	-	-	-	**90/90 (100%)**	**0/90** 0.0%

Analyte	Concentration (log_{10} copies/mL)	% Replicates Reported in Each Bin Result					Total Detected
		≥10^7	10^6	10^5	10^4	ND	
	None (No Analyte)	-	-	-	-	**1800/1800 (100%)**	**0/1800** 0.0%
Pseudomonas aeruginosa ATCC 10145	**7.0**	**90/90 (100%)**	-	-	-	-	**90/90** 100%
	6.0	20/90 (22.2%)	**70/90 (77.8%)**	-	-	-	**90/90** 100%
	5.0	-	24/90 (26.7%)	**66/90 (73.3%)**	-	-	**90/90** 100%
	4.0	-	-	16/90 (17.8%)	**74/90 (82.2%)**	-	**90/90** 100%
	3.0	-	-	-	14/90 (15.6%)	**76/90 (84.4%)**	**14/90** 15.6%
	2.0	-	-	-	-	**90/90 (100%)**	**0/90** 0.0%
	None (No Analyte)	-	-	-	-	**1800/1800 (100%)**	**0/1800** 0.0%
Serratia marcescens (OXA-48-like) GRE 1659005	**7.0**	**90/90 (100%)**	-	-	-	-	**90/90** 100%
	6.0	2/90 (2.2%)	**88/90 (97.8%)**	-	-	-	**90/90** 100%
	5.0	-	7/90 (7.8%)	**83/90 (92.2%)**	-	-	**90/90** 100%
	4.0	-	-	6/90 (6.7%)	**83/90 (92.2%)**	1/90 (1.1%)	**89/90** 98.9%
	3.0	-	-	-	6/90 (6.7%)	**84/90 (93.3%)**	**6/90** 6.7%
	2.0	-	-	-	-	**90/90 (100%)**	**0/90** 0.0%
	None (No Analyte)	-	-	-	-	**1800/1800 (100%)**	**0/1800** 0.0%
Staphylococcus aureus* subsp. *aureus (*mecA/C* and MREJ (MRSA)) ATCC 43300	**7.0**	**90/90 (100%)**	-	-	-	-	**90/90** 100%
	6.0	-	**90/90 (100%)**	-	-	-	**90/90** 100%
	5.0	-	-	**90/90 (100%)**	-	-	**90/90** 100%
	4.0	-	-	-	**89/90 (98.9%)**	1/90 (1.1%)	**89/90** 98.9%
	3.0	-	-	-	-	**90/90 (100%)**	**0/90** 0.0%
	2.0	-	-	-	-	**90/90 (100%)**	**0/90** 0.0%
	None (No Analyte)	-	-	-	2/1260 (0.2%)	**1258/1260 (99.8%)**	**2/1260** 0.2%
Streptococcus agalactiae ATCC 13813	**7.8**	**89/90 (98.9%)**	1/90 (1.1%)	-	-	-	**90/90** 100%
	6.8	**89/90 (98.9%)**	-	-	-	1/90 (1.1%)	**89/90** 98.9%
	5.8	-	**88/90 (97.8%)**	1/90 (1.1%)	1/90 (1.1%)	-	**90/90** 100%
	4.8	-	-	**89/90 (98.9%)**	1/90 (1.1%)	-	**90/90** 100%
	3.8	-	-	-	**86/90 (95.6%)**	4/90 (4.4%)	**86/90** 95.6%
	2.8	-	-	-	3/90 (3.3%)	**87/90 (96.7%)**	**3/90** 3.3%
	None (No Analyte)	-	-	-	-	**1800/1800 (100%)**	**0/1800** 0.0%
Streptococcus pneumoniae ATCC 6303	**6.5**	**90/90 (100%)**	-	-	-	-	**90/90** 100%
	5.5	-	**90/90 (100%)**	-	-	-	**90/90** 100%
	4.5	-	-	**89/90 (98.9%)**	1/90 (1.1%)	-	**90/90** 100%
	3.5	-	-	-	**89/90 (98.9%)**	1/90 (1.1%)	**89/90** 98.9%

Analyte	Concentration (log_{10} copies/mL)	% Replicates Reported in Each Bin Result					Total Detected
		≥10^7	10^6	10^5	10^4	ND	
	2.5	-	-	-	-	**90/90 (100%)**	**0/90** 0.0%
	1.5	-	-	-	-	**90/90 (100%)**	**0/90** 0.0%
	None (No Analyte)	-	-	-	-	**1800/1800 (100%)**	**0/1800** 0.0%
Streptococcus pyogenes ATCC 49399	**7.8**	**90/90 (100%)**	-	-	-	-	**90/90** 100%
	6.8	**90/90 (100%)**	-	-	-	-	**90/90** 100%
	5.8	5/90 (5.6%)	**84/90 (93.3%)**	1/90 (1.1%)	-	-	**90/90** 100%
	4.8	-	4/90 (4.4%)	**86/90 (95.6%)**	-	-	**90/90** 100%
	3.8	-	-	3/90 (3.3%)	**87/90 (96.7%)**	-	**90/90** 100%
	2.8	-	-	-	16/90 (17.8%)	**74/90 (82.2%)**	**16/90** 17.8%
	None (No Analyte)	-	-	-	-	**1800/1800 (100%)**	**0/1800** 0.0%

The precision of the antimicrobial resistance (AMR) genes was measured as the reproducibility of analyte detection on each system and overall, presented in Table 103 as the percent of replicates that are detected at concentrations of the associated bacterium that are within the reportable range, or below the reportable range, as well as the percent agreement with the expected Not Detected result in unspiked samples.

Table 103. Reproducibility of BIOFIRE Pneumonia Panel Antimicrobial Resistance Gene Results

AMR Gene Organism	Concentration of Organism (log_{10} copies/mL)	Expected Result	Agreement with the Expected Result			All Systems/Sites [95% CI]
			BIOFIRE Site A	BIOFIRE 2.0 Site B	BIOFIRE TORCH Site C	
CTX-M *Klebsiella oxytoca* GRE 1254054	**Reportable Range (3.5 – 7.5)**	**Detected**	**150/150** 100%	**149/150**[a] 99.3%	**150/150** 100%	**449/450**[a] **99.8%** [98.8%-99.9%]
	Below Reportable Range (2.5)	Detected (Variable)	**0/30** 0.0%	**1/30** 3.3%	**0/30** 0.0%	**1/90** **1.1%** [0.03%-6.0%]
	None (No Analyte)	N/A or Not Detected	**600/600** 100%	**599/600** 99.8%	**600/600** 100%	1799/1800 99.9% [99.7%-100%]
IMP *Escherichia coli* GRE 1062016	**Reportable Range (4.0 – 7.0)**	**Detected**	**120/120** 100%	**120/120** 100%	**120/120** 100%	**360/360** **100%** [99.0%-100%]
	Below Reportable Range (2.0-3.0)	Detected (Variable)	**10/60** 16.7%	**9/60** 15.0%	**3/60** 5.0%	**22/180** **12.2%** [7.8%-17.9%]
	None (No Analyte)	N/A or Not Detected	**600/600** 100%	**600/600** 100%	**600/600** 100%	**1800/1800** **100%** [99.8%-100%]
KPC *Klebsiella pneumoniae* AR-Bank#0097	**Reportable Range (4.0 – 7.0)**	**Detected**	**120/120** 100%	**119/120**[b] 99.2%	**120/120** 100%	**359/360**[b] **99.7%** [98.5%-100%]
	Below Reportable Range (2.0 – 3.0)	Detected (Variable)	**14/60** 23.3%	**12/60** 20.0%	**9/60** 15.0%	**35/180** **19.4%** [13.9%-25.0%]
	None (No Analyte)	N/A or Not Detected	600/600 100%	600/600 100%	600/600 100%	**1800/1800** **100%** [99.8%-100%]
***mecA/C* and MREJ (MRSA)** *Staphylococcus aureus* ATCC 43300	**Reportable Range (4.0 – 7.0)**	**Detected**	**119/120**[b] 99.2%	**118/120**[b] 98.3%	**120/120** 100%	**357/360**[b] **99.2%** [97.6%-99.8%]
	Below Reportable Range (2.0 – 3.0)	Detected (Variable)	**0/60** 0.0%	**0/60** 0.0%	**0/60** 0.0%	**0/180** **0%** [0.0%-2.0%]
	None	N/A or	**420/420**	**420/420**	**420/420**	**1260/1260**

AMR Gene Organism	Concentration of Organism (log_{10} copies/mL)	Expected Result	Agreement with the Expected Result			
			BIOFIRE Site A	BIOFIRE 2.0 Site B	BIOFIRE TORCH Site C	All Systems/Sites [95% CI]
	(No Analyte)	Not Detected	100%	100%	100%	**100%** [99.7%-100%]
NDM *Acinetobacter baumannii* AR-Bank#0033	**Reportable Range (3.5 – 7.5)**	**Detected**	**150/150** 100%	**149/150**[a] 99.3%	**150/150** 100%	**449/450**[a] **99.8%** [98.8%-100%]
	Below Reportable Range (2.5)	Detected (Variable)	**1/30** 3.3%	**1/30** 3.3%	**0/30** 0.0%	**2/90** **2.2%** [0.3%-7.8%]
	None (No Analyte)	N/A or Not Detected	**599/600** 99.8%	**600/600** 100%	**600/600** 100%	**1799/1800** **99.9%** [99.7%-100%]
OXA-48-like *Serratia marcescens* GRE 1659005	**Reportable Range (4.0 – 7.0)**	**Detected**	**120/120** 100%	**119/120**[b] 99.2%	**120/120** 100%	**359/360**[b] **99.70%** [98.5%-100%]
	Below Reportable Range (2.0 – 3.0)	Detected (Variable)	**14/60** 23.3%	**12/60** 20.0%	**9/60** 15.0%	**35/180** **19.4%** [13.9%-26.0%]
	None (No Analyte)	N/A or Not Detected	**598/600** 99.7%	**600/600** 100%	**600/600** 100%	**1798/1800** **99.9%** [99.6%-100%]
VIM *Enterobacter cloacae* AR-BANK#0154	**Reportable Range (4.0 – 7.0)**	**Detected**	**120/120** 100%	**120/120** 100%	**120/120** 100%	**360/360** **100%** [99.0%-100%]
	Below Reportable Range (2.0 – 3.0)	Detected (Variable)	**10/60** 16.7%	**9/60** 15.0%	**3/60** 5.0%	**22/180** **12.2%** [7.8%-17.9%]
	None (No Analyte)	N/A or Not Detected	**599/600** 99.8%	**600/600** 100%	**600/600** 100%	**1799/1800** 99.9% [99.7%-100%]

[a] CTX-M and NDM Not Detected results observed at the corresponding bacterial concentration of 4.5 log_{10} copies/mL.

[b] KPC, *mecA/C* and MREJ (MRSA), and OXA-48-like Not Detected results observed at the corresponding bacterial concentration of 4.0 log_{10} copies/mL.

Interference

Potentially interfering substances that could be present in BAL-like or sputum-like specimens or that may be introduced during specimen collection and testing were evaluated for their effect on BIOFIRE Pneumonia Panel performance. Substances included endogenous substances that may be found in specimens at normal or elevated levels (e.g. blood, mucus/mucin, human genomic DNA), various commensal or infectious microorganisms, medications, a variety of sample processing substances and substances used to clean, decontaminate, or disinfect work areas. The performance of the BIOFIRE Pneumonia Panel has not been established with all potentially interfering medications for the treatment of lower respiratory tract infections. The effect of interfering substances has only been evaluated for those listed in Table 104. Interference from substances that were not evaluated could lead to erroneous results.

Each substance was added to contrived samples containing representative qualitatively reported organisms and representative organisms with bin reporting. Qualitatively reported organisms were at concentrations near (2-3×) LoD and those with bin reporting were present at 4.0 log_{10} (copies/mL) (e.g. in the lowest reported bin). The concentration of substance added to the samples (Table 104) was equal to or greater than the highest level expected to be in BAL-like or Sputum-like specimens.

Four of the evaluated substances were found to interfere with the ability of the BIOFIRE Pneumonia Panel to report accurate analyte results; Bleach, MycoPrep, 2% NaOH, and 5% Oxalic acid. Each of these substances contain chemicals known to react with nucleic acids, altering their chemical structure. The interference observed was related to the inability to detect the chemically modified nucleic acids. Treatment of specimens with these substances prior to BIOFIRE Pneumonia Panel testing may result in loss of analyte detection, therefore samples that have been in contact with these substances should not be tested using the BIOFIRE Pneumonia Panel. None of the other substances tested were shown to interfere with the

BIOFIRE Pneumonia Panel results, however, testing of specimens that have been centrifuged or pre-treated by addition of enzyme, media, mucolytic agent, or decontaminating substances is not recommended.

Table 104. Evaluation of Potentially Interfering Substances on the BIOFIRE Pneumonia Panel

Substance	Concentration Tested	Testing Outcome
Endogenous Substances		
Blood	10% v/v	No Interference
Albumin	60 mg/mL	No Interference
HCl (gastric acid)	5 mmol/L	No Interference
Hemoglobin	2 mg/mL	No Interference
Human Cells (K-562 cell line)	3.8E+06 cells/mL	No Interference
Immunoglobulins (IgG)	60 mg/mL	No Interference
Mucin	16 mg/mL	No Interference
Exogenous Substances		
Albuterol (bronchodilator)	1.7 µmol/L	No Interference
Benzocaine (Orajel)	1.0 % w/v	No Interference
Epinephrine (hormone, bronchodilator)	8.3 µg/mL	No Interference
Galphimia glauca (Homeopathic remedy)	1.0 % w/v	No Interference
Guaifenesin (expectorant)	15.2 mmol/L	No Interference
Lidocaine	5.1 mmol/L	No Interference
Menthol and cetylpyridinium chloride (Cepacol Mouthwash)	1.0% v/v	No Interference
Mupirocin (antibiotic)	6.0 ng/mL	No Interference
Nicotine	6.2 µmol/L	No Interference
Pentamidine (antimicrobial)	1.5 mg/mL	No Interference
Phenylephrine hydrochloride (decongestant)	0.3 mg/mL	No Interference
Tobramycin sulfate (antibiotic)	30 mg/mL	No Interference
Zanamivir (influenza antiviral)	426 ng/mL	No Interference
Competitive Microorganisms		
Actinobacillus actinomycetecomitans	3.8E+07 CFU/mL	No Interference
Aspergillus fumigatus	5.5E+07 CFU/mL	No Interference
Burkholderia cepacia	1.7E+07 CFU/mL	No Interference
Cryptococcus neoformans	2.5E+05 CFU/mL	No Interference
Enterovirus D68	1.4E+06 copies/mL	No Interference
Haemophilus influenzae	1.8E+07 CFU/mL	No Interference
Legionella pneumophila	8.1E+06 CFU/mL	No Interference
Respiratory syncytial virus	3.5E+04 copies/mL	No Interference
Staphylococcus epidermidis	1.9E+07 CFU/mL	No Interference
Streptococcus mutans	5.9E+06 CFU/mL	No Interference
Streptococcus pyogenes	5.5E+06 CFU/mL	No Interference
Varicella zoster virus	8.7E+07copies/mL	No Interference
Disinfection/Cleaning Substances		
Reagent Alcohol	7.0%	No Interference
Bleach	1.0% v/v (600 ppm chlorine)	**Interference Observed** [a]
Sample Processing Materials[a]		
Copan Snotbuster (active ingredient DTT)	50.0% v/v	No Interference
Sputolysin (active ingredient DTT)	50.0% v/v	No Interference
SPUTASOL (active ingredient DTT + salts)	50.0% v/v	No Interference
MycoPrep (active ingredient NaOH + NALC)	50.0% v/v	**Interference Observed** [b]
NaOH (decontaminant)	1.0%	**Interference Observed** [b]
Oxalic Acid (decontaminant)	2.5%	**Interference Observed** [b]

[a] BIOFIRE Pneumonia Panel testing of lower respiratory specimens that have been processed or treated with these or other substances (e.g. trypsin) has not been validated and is not recommended.

[b] Pouch controls passed but Not Detected results were reported for one or more analytes after incubation of the sample with substance. Substance(s) are known to chemically interact with and damage nucleic acids (DNA and/or RNA) to prevent amplification.

APPENDIX A

Symbols Glossary

ISO 15223-1 Medical devices - Symbols to be used with medical devices labels, labeling and information to be supplied					
5.1.1 	Manufacturer	5.1.2 	Authorized representative in the European Community	5.1.4 	Use-By date (YYYY-MM-DD)
5.1.5 	Batch Code (Lot Number)	5.1.6 	Catalog Number	5.1.7 	Serial Number
5.2.8 	Do Not Use if Package Is Damaged	5.3.2 	Keep Away from Sunlight	5.3.7 	Temperature Limit
5.4.2 	Do Not Reuse	5.4.3 	Consult Instructions for Use	5.5.1 	In vitro Diagnostic Medical Device
5.5.5 	Contains Sufficient For <n> Tests			5.7.10 	Unique Device Identifier (UDI)
Use of Symbols in Labeling – 81 FR 38911, Docket No. (FDA-2013-N-0125)					
Rx Only	Prescription Use Only				
United Nations Globally Harmonized System of Classification and Labeling of chemicals (GHS) (ST/SG/AC.10/30)					
	Serious eye damage, Category 1		Acute toxicity, oral, Category 4 & Skin corrosion, irritation, Category 2		Acute aquatic hazard, Category 1 & Long-term aquatic hazard, Category 1
European Union In Vitro Diagnostic Directive (IVDD 98/79/EC) and European In Vitro Diagnostic Regulation (IVDR 2017/746)					
	European Union Conformity				
Manufacturer Symbols (BIOFIRE Diagnostics, LLC)					
	A BIOFIRE Pneumonia Panel product			BIOFIRE Pneumonia Panel	
	European Union Product Importer				

APPENDIX B

Contact and Legal Information

Customer and Technical Support	
Reach Us on the Web http://www.BIOFIREDX.com **Reach Us by Email** BioFireSupport@biomerieux.com **Reach Us by Mail** 515 Colorow Drive Salt Lake City, UT 84108 USA	**Reach Us by Phone** 1-800-735-6544 – Toll Free (801) 736-6354 – Utah **Reach Us by Fax** (801) 588-0507
Customer and Technical Support outside of the U.S.	
Contact the local bioMérieux sales representative or an authorized distributor for technical support.	

BIOFIRE Diagnostics, LLC
515 Colorow Drive
Salt Lake City, UT 84108
USA

Qarad EC-REP BV
Pas 257
B-2440 Geel, Belgium

bioMérieux SA
376, Chemin de l'Orme
69280 Marcy l'Etoile-France

NOTE FOR CUSTOMERS WITHIN THE EUROPEAN UNION (EU): Any serious incident that has occurred in relation to the device must be reported to BIOFIRE Diagnostics, LLC or local bioMérieux sales representative and the competent authority of the Member State in which the user and/or the patient is established.

Warranty Information

Product warranty information is available online at:

http://www.biofiredx.com/support/documents/

For warranty information for customers outside the United States, contact the local bioMérieux sales representative or an authorized distributor.

APPENDIX C

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REVISION HISTORY

Version	Revision Date	Description of Revision(s)
01-02	N/A	Previous Revisions
03	February 2021	Additions: • Revision history table Updates to: • Symbols Glossary • E-labeling links • Branding and logo • EC REP Address Removals: • 6-pack part number (RFIT-ASY-0145) as this configuration is no longer commercially available. • BIOFIRE FILMARRAY (1st generation system) references and operation. Please refer to revision 02 for information on the operation of the BIOFIRE Pneumonia Panel with the BIOFIRE FILMARRAY.
04	September 2021	Additions: • Limitation 17: There is an increased risk of false negative Adenovirus results for adenovirus species C when using a pouch that is within 6 months of the expiration date due to a 10-100 x loss in sensitivity (i.e. impairment leading to an increase in the LoD). The test performance is not impacted if kits are more than 6 months from expiration date. Performance for other adenovirus species is not impacted. • Limitation 18: If using a pouch that is within 6 months of expiration when a patient is suspected of adenovirus C infection, confirm all negative Adenovirus results using another method prior to reporting the result, or alternatively, do not report a negative Adenovirus result. Updates to: • Footnote added to Table 62 in the Limit of Detection section: [c] LoD for adenovirus species C is 10 – 100 x impaired when pouches are within 6 months of expiration (see Limitations). Removals: • Sample Buffer ampoule volume • Hydration Injection Vial volume
05	August 2022	Additions: • UDI Symbol to symbols glossary • Pneumonia Panel symbol to symbols glossary • Limitation 19: False positives and false negatives can be the result of a variety of sources and causes. It is important that results be used in conjunction with other clinical, epidemiological, or laboratory information. • Intended User and Use Environment • Safety Precautions section to provide additional information and warnings on the Sample Buffer. Note: This information was previously only contained within the SDS. • Note for Staphylococcus aureus mecA/C and MREJ interpretation was added in the 'Interpretations for Antimicrobial Resistance (AMR) Genes' section. Updates to: • Enterobacter cloacae complex (ECC) member information updated in the 'Summary of Detected Organisms' section and the following analytical tables: Table 76, Table 98, and Table 99. • MREJ footnote added to the following analytical reactivity tables: Table 63, Table 90, and Table 91 • Minor typographical errors and minor wording changes for consistency and clarity. • Updated pathogen list to reflect new capitalization. Removals: • "DO NOT REFRIGERATE" from Reagent Storage, Handling, and Stability section • Note: BIOFIRE's 1st generation system, the BIOFIRE® FILMARRAY® (REF: FLM1-ASY-0001), is no longer being distributed or manufactured. For information on the operation of this system with the BIOFIRE Pneumonia Panel, please refer to revision 02 of this Instructions for Use

Version	Revision Date	Description of Revision(s)
06	August 2023	Updates to: • Minor typographical errors and minor word changes for consistency and clarity. • Branding • Additional Sample Buffer ampoule steps • Customer Technical Support Email

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