BIOFIRE® FILMARRAY® Meningitis/Encephalitis (ME) Panel

Instructions for Use	www.biofiredx.com/e-labeling/ITI0035
Quick Guide	www.biofiredx.com/e-labeling/ITI0012
Safety Data Sheet (SDS)	www.biofiredx.com/e-labeling/ITI0067
Panel Software	www.biofiredx.com/e-labeling/ITIFA20ME14
Summary of Safety and Performance (Applicable for EU Customers)	http://www.biofiredx.com/e-labeling/ITIME4815

Rx Only

UK CA 0086

IVD

CE 2797

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

INTENDED PURPOSE

Intended Use

The BIOFIRE® FILMARRAY® Meningitis/Encephalitis (ME) Panel is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with BIOFIRE® FILMARRAY® Systems. The BIOFIRE ME Panel is capable of simultaneous detection and identification of multiple bacterial, viral, and yeast nucleic acids directly from cerebrospinal fluid (CSF) specimens obtained via lumbar puncture from individuals with signs and/or symptoms of meningitis and/or encephalitis. The following organisms are identified using the BIOFIRE ME Panel:

Bacteria:
- *Escherichia coli* K1
- *Haemophilus influenzae*
- *Listeria monocytogenes*
- *Neisseria meningitidis* (encapsulated)
- *Streptococcus agalactiae*
- *Streptococcus pneumoniae*

Viruses:
- Cytomegalovirus
- Enterovirus
- Herpes simplex virus 1
- Herpes simplex virus 2
- Human herpesvirus 6
- Human parechovirus
- Varicella zoster virus

Yeast:
- *Cryptococcus neoformans/gattii*

The BIOFIRE ME Panel is indicated as an aid in the diagnosis of specific agents of meningitis and/or encephalitis and results are meant to be used in conjunction with other clinical, epidemiological, and laboratory data. Results from the BIOFIRE ME Panel are not intended to be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results do not rule out co-infection with organisms not included in the BIOFIRE ME Panel. The agent detected may not be the definite cause of the disease. Negative results do not preclude central nervous system (CNS) infection. Not all agents of CNS infection are detected by this test and sensitivity in clinical use may differ from that described in the package insert.

The BIOFIRE ME Panel is not intended for testing of specimens collected from indwelling CNS medical devices.

The BIOFIRE ME Panel is intended to be used in conjunction with standard of care culture for organism recovery, serotyping, and antimicrobial susceptibility testing.

Intended User and Use Environment

The BIOFIRE ME 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

Central nervous system (CNS) infections are responsible for causing inflammatory conditions of the brain and/or meningeal tissues surrounding the brain (i.e., meningitis, encephalitis, meningoencephalitis; here collectively termed ME). Approximately 15% of cases are fatal and many other cases result in life-long disabilities such as loss of limbs, visual and hearing deficits, seizures, and altered learning and memory.[1] The BIOFIRE ME Panel conducts tests for the identification of 14 potential CNS pathogens from CSF (Table 1). The specimen can be tested using the BIOFIRE ME Panel with results available within about one hour.

Table 1. Bacteria, Viruses, and Yeast Detected by the BIOFIRE ME Panel

Bacteria	
Escherichia coli K1	*Neisseria meningitidis*
Haemophilus influenzae	*Streptococcus agalactiae*
Listeria monocytogenes	*Streptococcus pneumoniae*
Viruses	
Cytomegalovirus (CMV)	Enterovirus (EV)
Human herpesvirus 6 (HHV-6)	Herpes simplex virus 1 (HSV-1)
Human parechovirus (HPeV)	Herpes simplex virus 2 (HSV-2)
Varicella zoster virus (VZV)	
Yeast	
Cryptococcus neoformans/gattii	

Summary of Detected Organisms

Bacteria

***Escherichia coli* K1** strains account for nearly 80% of *E. coli* isolated from CSF.[2] While most *E.coli* are harmless enteric organisms residing in the intestines of humans and animals, some cause gastrointestinal illness and extra-intestinal infections (e.g. urinary tract infections, bacteremia, and meningitis). *E. coli* associated with meningitis contain virulence factors which contribute to their pathogenesis by allowing them to spread through the blood, hijack normal host cell functions, infiltrate endothelial cells, and gain access to the tissues of the CNS.[3] The K1 antigen is a capsule that protects the bacteria from the immune system. These infections are of particular concern for pre-term babies and neonates, and are responsible for nearly 45% or 30% of meningitis cases in these age groups with a mortality rate of 13 or 25%, respectively.[4] Infections in adults are less common and generally opportunistic in nature following exposure of sterile organs to contents of the gastrointestinal tract following trauma or surgical procedures; the mortality rate for adults is reported to be 28-36%.[5,6]

Haemophilus influenzae is a gram-negative coccobacillus that is isolated exclusively from humans.[7] Strains of *H. influenzae* are divided into two groups based on the presence or absence of a polysaccharide capsule.[7,8] 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,[7] with a mortality rate of 3 to 6% and a further 20 to 30% developing permanent sequelae ranging from mild hearing loss to mental retardation.[8] In areas with routine vaccination, the majority of invasive *H. influenzae* infections are caused by non-typeable strains and remain an important cause of meningitis particularly for persons with predisposing conditions such as otitis or sinusitis, diabetes, immune deficiency, or head trauma with CSF leakage.[9] Meningitis due to *H. influenzae* occurs at an estimated rate of approximately 0.08 cases per 100,000 in the United States,[1] and has been reported as the etiologic agent of bacterial meningitis in 20-50% of cases worldwide over the last several decades.[10]

Listeria monocytogenes, the causative agent of listeriosis, is a gram-positive bacillus that is ubiquitous in soil and water and can be found in the gastrointestinal tract of up to 5% of healthy human adults.[11,12] Listeriosis is considered one of the most severe bacterial foodborne infections due to its high mortality rate even with early antibiotic treatment (11 – 60%).[12,13]

Invasive listeriosis can result in abortion, sepsis, meningitis, and meningoencephalitis. Populations at risk for developing invasive listeriosis include the immunosuppressed, pregnant women, neonates, fetuses, and the elderly.[2,11] Meningitis due to *L. monocytogenes* is reported to be approximately 0.05 cases per 100,000 persons in the US per year,[1] and causes from 0.5-2.0% of bacterial meningitis cases in non-U.S. countries.[10]

***Neisseria meningitidis* (Encapsulated)** is a fastidious, aerobic, gram-negative diplococcus that is transmitted by contact with mucus or respiratory droplets, often from asymptomatic carriers. There are at least 12 different serogroups of *N. meningitidis,* six of which are associated with epidemics (groups A, B, C, W, X, and Y).[14] The serogroup refers to types of capsular antigens. Although unencapsulated *N. meningitidis* has not historically been recognized as pathogenic, clinical data has demonstrated that unencapsulated strains of *N. meningitidis* can cause disease in both immunocompetent and immunocompromised patients.[15] Meningococcal disease (spinal meningitis and/or meningococcemia) is rare in developed countries but can occur in outbreaks and is still a public health issue in developing countries. It is most common in infants, children, and young adults, and appears in places with crowded living conditions (e.g., college dormitories and military barracks). Seasonal incidence peaks in late winter and early spring[16] with an annual incidence of about 0.2 cases per 100,000 in the US.[1] The disease can progress extremely quickly (<24 hours) with hypotension, multi-organ dysfunction, shock, peripheral ischemia, and limb loss and has a mortality rate of approximately 5-10%.[17] There are six licensed meningococcal vaccines available in US that may be used in persons of all ages, depending on the vaccine.[18] Despite extensive vaccination efforts worldwide, several serogroups of *N. meningitidis* still cause seasonal outbreaks, particularly in sub-Saharan Africa.[14] Extreme reductions in serogroup C meningococcal meningitis have been observed in countries where vaccines providing protection for this serogroup have been introduced.[19]

Streptococcus agalactiae (Group B *Streptococcus* or GBS) is an important cause of meningitis in neonates, particularly those that are pre-term, and is often coincident with neonatal sepsis.[2,20] The most important risk factor for neonatal disease is maternal colonization with GBS.[2] Since 1996, CDC guidelines (updated in 2010)[21] have called for prophylactic antibiotic treatment several hours before delivery and have resulted in declining rates of neonatal GBS.[22] In adult patients, GBS is associated with advanced age or severe underlying health conditions. Overall incidence in the U.S. is estimated to be 0.25 infections per 100,000[1] and neonatal GBS disease has ranged from 0.2-2.4 per 1,000 births in Europe over the last few decades[23]. Mortality rates range from 10% for neonates[24] to 25-30% in adults.[25,26]

Streptococcus pneumoniae colonizes the upper respiratory tract and is the most frequently isolated respiratory pathogen in community-acquired pneumonia. It is also a major cause of meningitis, particularly in pediatric and elderly patients, and especially in those with underlying medical conditions, with an incidence rate of approximately 0.8 infections per 100,000 in the US,[1] and causes 20-31% of bacterial meningitis cases in non-U.S. countries.[10] The mortality rate is also high: 8-15% for children and 20-37% for adults.[27] Mortality approaches 50% in resource-poor countries, especially where HIV co-infection is a factor.[28] Neurological sequelae (cognitive impairment, deafness, epilepsy) are reported for up to 40% of survivors.[29,30] 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[31] and help reduce the risk of both invasive disease and pneumococcal pneumonia by 50-80%.[32]

Viruses

Human **cytomegalovirus** (CMV) is a double stranded DNA virus of the *Herpesviridae* family. Seroprevalance data show that infection is nearly ubiquitous in the population world-wide, with rates approaching 100% in developing countries[33] and 36-90% in the US depending on age and race/ethnicity.[34] Maternal transfer of CMV may result in congenital infection with serious long-term sequelae, but generally infections are largely unnoticed in healthy individuals or may present mononucleosis-like illness. While severe illness in immunocompetent patients is rare,[35] CMV is an opportunistic pathogen in immunocompromised or immunosuppressed individuals, either as an initial infection or activation of a latent infection. Until the 1990s, before the availability of highly-active antiretroviral therapy, it is estimated that nearly half of HIV-infected

patients developed severe CMV infection, primarily CMV retinitis, colitis or pneumonia.[36] Left untreated, CMV disease can be fatal in these populations.

Enteroviruses (EV) are small RNA viruses that are members of the *Picornaviridae* family and are associated with human illnesses ranging from asymptomatic or mild infections to serious CNS illnesses requiring hospitalization. Infection rates are highest in children, with the majority of infections occurring during summer months.[37] The most common EV serotypes are coxsackieviruses A9 and B1, and echoviruses 6, 9, and 18, which account for over 50% of serotyped detections. [37] Infections are spread via fecal-oral and respiratory routes and can spread quickly in community settings, particularly in areas with poor sanitation.[38] EV is one of the commonly identified causes of infectious encephalitis/meningitis, with prevalence rates reported between 5.5-30% depending on location and patient demographics.[39–41]

Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) are DNA viruses of the *Herpesviridae* family named for the spreading skin ulcerations caused by infection with these viruses. HSV-1 infections usually occur early in childhood and manifest primarily as oral lesions, whereas HSV-2 is primarily associated with genital lesions and infections are acquired later in life and are associated with sexual activity. HSV establishes residency in nerve cells following initial infection (which is asymptomatic in most cases). Viral activation resulting in lesions or other severe disease outcomes (such as CNS infection), may occur throughout life, and are associated with fever, injury, exposure to UV irradiation (sunlight), emotional stress, hormone irregularities, and changes in immune status.[38] In the U.S., overall seroprevalence for HSV-1 is around 60%.[42] The overall seroprevalence for HSV-2 is around 16% but varies with age, sex, and ethnicity.[43] Worldwide, it is estimated that ~90% of people are infected with HSV-1, and HSV-2 is less common with 15-80% of people infected.[44] HSV is one of the most common causes of viral encephalitis, and is a significant cause of meningitis. In a large study of over 1600 CSF specimens in the United Kingdom,[45] HSV-1 was found in 25 (1.5%) patients (almost all of whom had encephalitis) and HSV-2 was found in 33 (1.9%) patients (almost all of whom had meningitis). This overall prevalence of ~3% in CSF is similar to that seen in a recent study of CSF patients in New York State.[41] This study also saw a similar distribution of HSV-1 and HSV-2 in encephalitis versus meningitis.

Human herpesvirus 6 (HHV-6) was discovered in the mid-1980s,[46] when the rise of immunocompromised patients led to an increase in the population susceptible to severe disease outcome.[38] There are two species of the virus: HHV-6A and HHV-6B. Studies have shown that over 95% of persons over the age of two are positive for one or both variants[47] and the infection establishes latency due to viral integration into host cells. Chromosomally integrated HHV-6 (ciHHV-6) in somatic cells allows persistence and sporadic reactivation. Integration into the germ cell line allows vertical transmission, called inherited ciHHV-6 (iciHHV-6).[48,49] While primary infection with HHV-6B causes roseola rash in infants, the clinical manifestations of primary infection with HHV-6A remain somewhat undefined; however, some studies have suggested that HHV-6A infection may be linked to inflammatory or neurological disease, and that HHV-6A may have an increased neurotropism compared to HHV-6B.[50,51] This hypothesis is supported by the finding that HHV-6 inhabits CNS tissues, including the brain,[52] where it may cause tissue damage leading to encephalitis/meningitis. Furthermore, HHV-6 was identified in CSF of 1.8% of patients with encephalitis/meningitis in a recent study.[41] CNS disease associated with HHV-6 is found in both children and adults, suggesting CNS invasion during primary infection is possible.[47] While immunocompetent patients may experience CNS infection, it is much more common in severely immunosuppressed individuals.[38,47] However, HHV-6 is known to reactivate in asymptomatic patients and can be detected by PCR in otherwise healthy individuals without signs of active HHV-6 infection.[53] Studies of HHV-6 in normal brain tissue have also identified HHV-6 DNA via PCR in up to 85% of patients without signs of active infection[54] and HHV-6 DNA may persist in the CSF after acute infection. In a study of 56 allogeneic stem cell transplant patients, HHV-6 DNA was detected in the CSF of 14 (27%) patients without CNS symptoms.[55] Given the prevalence of latent infection and potential for asymptomatic reactivation, positive HHV-6 results should be carefully interpreted in association with clinical symptoms and supplemental laboratory testing, especially in patients who are confirmed carriers of the virus or directly related to someone who is a confirmed carrier.[56]

Human parechoviruses (HPeV) comprise another genus of the *Picornaviridae* family. HPeV were originally classified as Enterovirus upon their discovery in the 1950s and at least a dozen serotypes have been identified. Seroprevalence for HPeV-1 approaches 100% in adult populations, with most infections occurring during early childhood.[57–59] As with EV, infections are spread via fecal-oral and respiratory routes with the most common symptoms being mild respiratory or gastrointestinal illness.[57] CNS disease from HPeV-1 is rare, but HPeV-3 is associated with severe disease outcomes such as sepsis, encephalitis, meningitis, and hepatitis in children <3 months of age.[60] Recent studies of CSF from infants with suspected CNS illness or sepsis have demonstrated HPeV at a prevalence of 3-17%, nearly all of which were HPeV-3.[61–63] Magnetic resonance imaging studies of infants who survive HPeV CNS disease show damage to white matter of the brain and developmental disabilities later in life.[64]

Varicella zoster virus (VZV) is a double stranded DNA virus of the *Herpesviridae* family that usually causes infections in childhood (chicken pox) and establishes latent presence in cells that can re-activate later in life (adult-onset zoster or shingles). VZV is primarily spread via aerosolization of viral particles from an infected individual, and infection of new hosts begins within the epithelial cells of the respiratory tract. Following primary infection (fever and malaise accompanied with a maculopapular rash), VZV establishes itself in the sensory ganglia of the nervous system where it remains latent.[38] In the US, nearly 90% of the population had been infected with VZV before the advent of vaccines.[38] Similar rates have been reported in European countries.[65,66] Of those infected, between 10-30% develop zoster (a painful rash along the dorsal ganglia), primarily later in life.[67,68] It is estimated that the median global incidence of zoster is 4.0-4.5 per 1,000 person-years,[69] which highlights the frequency of VZV re-activation worldwide. Studies have shown that VZV is transiently detectable by PCR in the blood of older, asymptomatic individuals (both immunocompetent and immunocompromised), suggesting reactivation occurs throughout life but is usually managed by the immune system.[70,71] Encephalitis and meningitis are complications of both varicella and zoster infections. In one study, VZV was the third most detected virus among patients with signs and symptoms of encephalitis/meningitis, with a reported prevalence of 1.9% in the study population.[41] For immunocompromised patients, VZV neuronal illness can become chronic and lead to progressive deterioration and death.[67] There are two live, attenuated VZV vaccines licensed for use in the US; one is for the vaccination of children against varicella and the other for zoster in older adults.[72]

Yeast

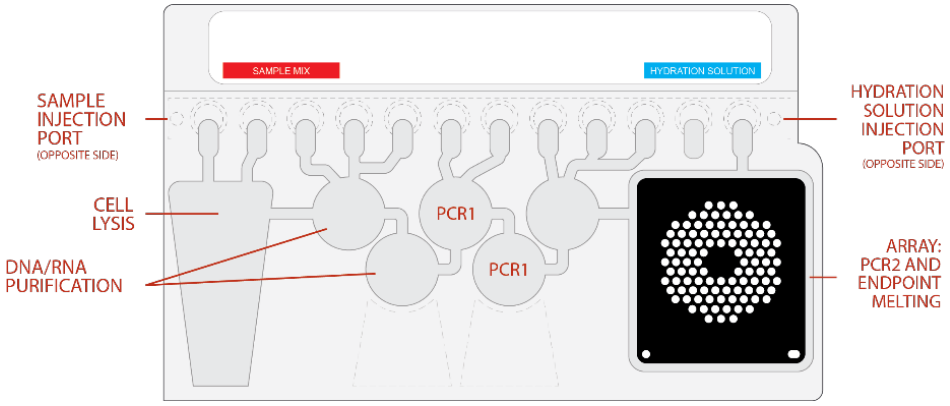
Cryptococcus neoformans and Cryptococcus gattii are pathogenic fungi found in soil and bird droppings that can become pathogenic following inhalation and spread to other organ systems (particularly the brain and meninges). *C. neoformans* is considered an opportunistic pathogen of immunocompromised individuals. It is the AIDS-defining illness in up to 50% of AIDS patients.[2,73] *C. gattii* infections are relatively rare but appear to be increasing. While typically associated with tropical and subtropical climates, since the 1990s *C. gattii* infections have been reported in British Columbia, Canada, the U.S. Pacific Northwest region, the Northeastern US, and in Europe.[74–77] In addition to those with reduced immune function, *C. gattii* can also cause disease in the immunocompetent, particularly in persons with underlying health conditions.[2] Mortality from cryptococcal meningitis is high, ranging from 10% to nearly 50% in immunocompromised patients.[73,76].

PRINCIPLE OF THE PROCEDURE

The BIOFIRE ME Panel pouch is a closed system disposable that stores all the necessary reagents for sample preparation, polymerase chain reaction (PCR), and detection in order to isolate, amplify, and detect nucleic acid from multiple meningitis and encephalitis pathogens within a single CSF specimen obtained from a lumbar puncture. After sample collection, the user injects hydration solution and sample combined with BIOFIRE® Sample Buffer into the pouch, places the pouch into a BIOFIRE® FILMARRAY® Module, and starts a run. The entire run process takes about an hour. Additional detail can be found in the appropriate BIOFIRE® System Operator's Manual.

During a run, the BIOFIRE System:

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



MATERIALS PROVIDED

Each kit contains sufficient reagents to test 30 (30-test kit; RFIT-ASY-0118) or 6 (6-test kit; RFIT-ASY-0119) samples:

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

  This software is required to run the BIOFIRE ME Panel and can be downloaded at www.biofiredx.com/e-labeling/ITIFA20ME14 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 System including accompanying system-specific core software
  - BIOFIRE® Pouch Loading Station
- 10% bleach solution or 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 ME Panel in conjunction with a patient's signs and symptoms, results from other diagnostic tests, and any relevant epidemiological information.
3. BIOFIRE ME Panel pouches are only for use with BIOFIRE 2.0 and BIOFIRE Torch systems.
4. Always check the expiration date on the pouch.
5. Do not use a pouch after its expiration date.
6. BIOFIRE pouches are stored under vacuum in individually wrapped canisters. To preserve the integrity of the pouch vacuum for proper operation, be sure that a BIOFIRE 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 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[78]
   - the CLSI Document M29 Protection of Laboratory Workers from Occupationally Acquired Infections[79]
   - Other appropriate guidelines
3. Follow your institution's safety procedures for handling biological samples.
4. Dispose of materials used in this assay, including reagents, samples, and used buffer vials, according to federal, state, and local regulations.
5. Sample Buffer contains Guanidinium chloride and Triton X100. The following statements apply:

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
        - P391 - Collect spillage.
        - 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 ME Panel Safety Data Sheet (SDS) for more information: https://www.biofiredx.com/e-labeling/ITI0067.

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

⚠ CAUTION: Never add Bleach to Sample Buffer or sample waste.

7. 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. The following first aid measures are recommended.
    - 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 ME Panel, it is important to guard against contamination of the specimen and work area by carefully following the testing process outlined in this instruction document, including these guidelines:

   - Clinical and laboratory personnel may carry or shed common pathogens (e.g. *S. pneumoniae*, *H. influenzae*, HSV-1, etc.) symptomatically or asymptomatically and can inadvertently contaminate the specimen while it is being collected, transported, or tested. Careful adherence to the sample handling and testing procedures and precautions described in this document is recommended to minimize the risk of contamination that could lead to erroneous test results. Precautions may include additional PPE, such as a face mask, when experiencing signs or symptoms of a respiratory infection or cold sore.
   - Samples should be processed in a clean biosafety cabinet if available, or according to local laboratory guidelines. 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 can be used when preparing samples .
   - A biosafety cabinet that is used for performing CSF pathogen testing (e.g. culture) should not be used for sample preparation or pouch loading.
   - Prior to processing samples, 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.

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 ME pouch is a closed system, the risk of amplicon contamination is low, provided that pouches remain intact after the test is completed. Adhere to the following guidelines to prevent amplicon contamination:

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

⚠**CAUTION:** 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 in the United States

Local, state, and federal regulations for notification of reportable disease are continually updated and include a number of organisms for surveillance and outbreak investigations.[80,81] Additionally, the Centers for Disease Control (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 their state and/or local regulations and should consult their local and/or state public health laboratories 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. Always check the expiration date and do not use reagents beyond the expiration date printed on the pouch or kit.
4. 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.
5. 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).
6. 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

This section describes the requirements for specimen collection, preparation, and handling that will help ensure accurate test results.

CSF Specimen Collection	CSF specimens should be collected via lumbar puncture, and should not be centrifuged, diluted, or otherwise processed prior to testing.
Minimum Sample Volume	0.2 mL (200 µL) of CSF
Transport and Storage	Specimens should be tested with the BIOFIRE ME Panel as soon as possible.[a] If storage is required, specimens can be held: • At room temperature (approximately 15-25°C) for up to one day • or refrigerated (approximately 2-8°C) for up to seven days.

[a] The performance validation included the evaluation of clinical specimens that were frozen at -70°C or lower for up to 127 days. However, longer frozen storage may be acceptable. Please follow your institutions rules and protocols regarding sample storage validation.

PROCEDURE

Refer to the BIOFIRE ME Panel Quick Guide or the appropriate BIOFIRE System Operator's Manual for more detail and pictorial representations of these instructions.

Gloves and other Personal Protective Equipment (PPE) should be used when handling pouches and samples. Only one BIOFIRE ME Panel pouch should be prepared at a time. Once sample is added to the pouch, it should be promptly transferred to the BIOFIRE Module to start the run. After the run is complete, the pouch should be discarded in a biohazard container.

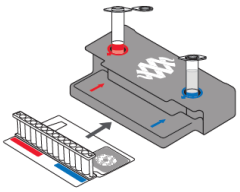
There is a risk of false positive results due to contamination of the specimen or testing area with organisms, their nucleic acids, or amplified product. Particular attention should be given to the Laboratory Precautions noted under the Warnings and Precautions section.

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.

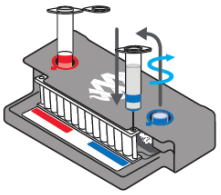
Note: The pouch may still be used even if the vacuum seal of the pouch is not intact. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.

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 in the red well of the Pouch Loading Station.
6. Place a blue-capped Hydration Injection Vial in 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.



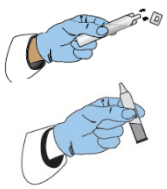
4. Forcefully push down in a firm and quick motion to puncture seal until a faint "pop" is heard and there is an ease in resistance. Wait as the correct volume of Hydration Solution is pulled into the pouch by vacuum.
    - If the hydration solution is not automatically drawn into the pouch, repeat Step 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 port was broken or retrieve a new pouch and repeat from Step 2 of the Prepare Pouch section.

Step 3: Prepare Sample Mix

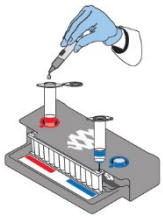
1. Add the Sample Buffer to the Sample Injection Vial.
    - Hold the Sample Buffer ampoule with tip facing up.

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 top: Firmly pinch the 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.



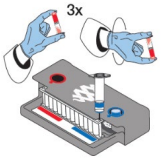
NOTE: Avoid squeezing the ampoule additional times. This will generate foaming, which should be avoided.

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

2. Thoroughly mix the patient specimen.
3. Using the transfer pipette provided in the test kit, draw cerebrospinal fluid (CSF) specimen to the second line (approximately 0.2 mL) of the transfer pipette.
4. Add the specimen to the Sample Buffer in the Sample Injection Vial.
5. Tightly close the lid of the Sample Injection Vial and discard the transfer pipette in a biohazard waste container.

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

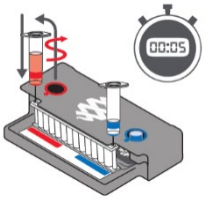
6. Remove the Sample Injection Vial from the Pouch Loading Station and gently invert the vial at least 3 times to mix.
7. Return the Sample Injection Vial to the red well of the Pouch Loading Station.



Step 4: Load Sample Mix

1. Slowly twist to unscrew the Sample Injection Vial from the red cap so it loosens from its 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 the red cap in the well of the Pouch Loading Station, and insert the Sample Injection Vial cannula tip into the pouch sample port located directly below the red arrow of the Pouch Loading Station.
3. Forcefully push down in a firm and quick motion to puncture seal (a faint “pop” is heard) and sample is pulled into the pouch by vacuum.
3. 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 the Prepare Pouch section.
4. Discard the Sample Injection Vial and the Hydration Injection Vial in an appropriate biohazard sharps container.
5. Record the Sample ID in the provided area on the pouch label (or affix a barcoded Sample ID) and remove the pouch from the Pouch Loading Station.

Step 5: Run Pouch

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

BIOFIRE 2.0

1. Ensure that the BIOFIRE system (module[s] and computer) is powered on and the software is launched.
2. Follow on-screen instructions and procedures described in the Operator's Manual to place the pouch in a module, enter pouch, sample, and operator information.
3. Pouch identification (Lot Number and Serial Number), Pouch Type, and Protocol are preprogrammed in the rectangular barcode located on the BIOFIRE pouch. The information will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, Pouch Type and Protocol can be manually entered from the information provided on the pouch label into the appropriate fields. 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 ME Panel pouch.

4. Enter the Sample ID. The Sample ID can be entered manually or scanned in by using the barcode scanner when a barcoded Sample ID is used.
5. If necessary, select and/or confirm the appropriate protocol for your sample type from the Protocol drop down list.
6. Enter a username and password in the Name and Password fields.

   Start the run.

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, Start run.

   Once the run has started, the screen displays a list of the steps being performed by the Module 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 the pouch in a biohazard container.
9. The run file is automatically saved in the BIOFIRE Software database and the test report can be viewed, printed, and/or saved as a PDF file.

BIOFIRE TORCH

1. Ensure that the BIOFIRE Torch System is on.
2. Select an available Module on the touch screen or scan the barcode on the pouch using the barcode scanner.
3. Pouch identification (Lot Number and Serial Number), Pouch Type and Protocol are preprogrammed in the rectangular barcode located on the pouch. The information will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, Pouch Type and Protocol can be manually entered from the information provided on the pouch label into the appropriate fields. 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 ME 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 Module.

   Ensure that the pouch fitment label is lying flat on top of pouch and not folded over. As the pouch is inserted, the Module will grab onto the pouch and pull it into the chamber.
6. If necessary, select and/or confirm a protocol from the protocol drop down list.
7. Enter operator user name 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 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 Software 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, 1st stage PCR, dilution, 2nd stage PCR and DNA melting. A positive control result indicates that all steps carried out in the BIOFIRE ME pouch were successful.
2. **PCR2 Control**
   The PCR2 Control assay detects a DNA target that is dried into wells of the array along with the corresponding primers. A positive result indicates that 2nd stage PCR was successful.

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

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 PCR2 Control is outside an acceptable range (79.8-83.8 for the RNA Process Control and 73.7-77.7 for the PCR2 Control). If required by local, state, or accrediting organization quality control requirements, users can monitor the system by trending Tm values for the control assays and maintain records according to standard laboratory quality control practices.[82,83] Refer to the appropriate BIOFIRE System Operator's Manual for instructions on obtaining control assay Tm values. The PCR2 Control is used in most BIOFIRE pouch types and can therefore be used to monitor the system when multiple pouch types are used on the same BIOFIRE System.

External Controls

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

INTERPRETATION OF RESULTS

The BIOFIRE software automatically analyzes and interprets assay results and displays the final results in a test report (see the BIOFIRE ME Panel Quick Guide to view an example of a test report). The analyses performed by the BIOFIRE software and details of the test report are described below.

Assay Interpretation

When 2nd stage PCR is complete, the BIOFIRE module performs a high resolution 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 2nd stage PCR array to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature I of the curve. The Tm value is then compared against the expected Tm range for the assay. If the software determines that the melt curve is positive and the Tm falls inside the assay-specific Tm range, the melt curve is called positive. If the software determines that the melt curve is negative or is not in the appropriate Tm range, the melt curve is called negative.

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

Organism Interpretation

The reported BIOFIRE ME Panel organism results (Detected or Not Detected) are based on analysis and interpretation of a single assay (most organisms) or a combination of two assays (*Haemophilus influenzae*, Herpes simplex virus 2 and Varicella zoster virus). For results that rely on two assays, a Detected result is reported when either one or both assays are positive and a Not Detected result is reported only when both assays are negative.

NOTE: Non-K1 E. coli serotypes may be present in a specimen and will not be detected by the BIOFIRE ME Panel.

NOTE: Non-encapsulated strains of Neisseria meningitidis are not detected by the BIOFIRE ME Panel.

NOTE: The BIOFIRE ME Panel does not distinguish between latent and active CMV and HHV-6 infections. Detection of these viruses may indicate primary infection, secondary reactivation, or the presence of latent virus. Results should always be interpreted in conjunction with other clinical, laboratory, and epidemiological information.

NOTE: Patients with a suspicion of cryptococcal meningitis and a negative cryptococcal PCR result, such as by the BIOFIRE ME Panel, should be tested for CrAG.

BIOFIRE ME Panel Test Report

The BIOFIRE ME Panel test report is automatically displayed upon completion of a run and contains three sections, the Run Summary, the Result Summary, and the Run Details (see the BIOFIRE Meningitis/Encephalitis Panel Quick Guide to view an example of a test report). The test report can be saved as a PDF or printed.

The **Run Summary** section of the test report provides the Sample ID, time and date of the run, control results and an overall summary of the test results. Any organism with a Detected result will be listed in the corresponding field of the summary. If all of the organism assays were negative, then None will be displayed in the Detected field. Controls are listed as Passed, Failed or Invalid. See the Controls Field section below for detailed information about the interpretation of controls and appropriate follow-up in the case of control failures.

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

The **Run Details** section provides additional information about the run including: pouch information (type, lot number, and serial number), Run Status (Completed, Incomplete, Aborted, Instrument Error, Instrument Communication Error, or Software Error), the protocol that was used to perform the test, the identity of the operator that performed the test, and the instrument module used to perform the test.

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

Controls Field

The Controls field on the test report will display Passed, Failed, or Invalid. The Controls field will display Passed only if the run completed successfully (no instrument module or software errors) and both of the pouch control assays (RNA Process Control and PCR2 Control) were successful. The Controls field will display Failed if the run was completed successfully (no instrument module or software errors) but one or both of the pouch control assays failed. If the control result is Failed, then the result for all of the tests on the panel are displayed as Invalid and the sample will need to be retested with a new pouch.

Table 2 provides a summary and explanation of the possible control results and follow-up actions.

Table 2. Interpretation of Controls Field on the BIOFIRE ME Panel Test Report

Control Result	Explanation	Action Required	Outcome
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 PCR2 Control) failed.	Repeat the test using a new pouch.	Accept the results of the repeat testing. If the error persists, contact Customer Technical Support for further instruction.

Control Result	Explanation	Action Required	Outcome
Invalid	The controls are invalid because the run did not complete. (Typically this indicates a software or hardware error).	Note any error codes displayed during the run and the Run Status field in the Run Details section of the report. Refer to the appropriate BIOFIRE System Operator's Manual or contact Technical Support for further instruction. Once the error is resolved, repeat the test or repeat the test using another instrument/Module.	Accept the valid results of the repeat testing. If the error persists, contact Customer Technical Support for further instruction.

Result Summary

The Result Summary section provides a complete list of the test results. Possible results for each organism include Detected, Not Detected, and Invalid. Table 3 provides an explanation for each interpretation and any follow-up necessary to obtain a final result.

Table 3. 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) associated with the interpretation were positive based on the following requirements for at least 2 of the 3 assay replicates: -a positive melt curve, and -the Tm for the melt data were within the assay specific limits, and -the Tm for the melt data were within 1°C of each other.	Report results. NOTE: If Detected results are reported for 2 or more organisms in a specimen, a retest of the specimen is recommended to confirm the polymicrobial result.
Not Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) associated with the interpretation were negative (did not meet the requirements for a positive assay described in Detected).	Report results.
Invalid	The run did not complete successfully (Aborted, Incomplete, Instrument Communication Error, Instrument Error, or Software Error) OR The pouch controls were not successful (Failed)	See Table 2, *Interpretation of Controls Field on BIOFIRE ME Panel Report,* for instruction.

LIMITATIONS

1. For prescription use only.
2. Results from this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
3. This test is a qualitative test and does not provide a quantitative value for the organism(s) nucleic acids detected in the specimen.
4. The BIOFIRE ME Panel is intended for use only on the BIOFIRE 2.0 and BIOFIRE Torch systems. Performance of the panel was established on the BIOFIRE FILMARRAY System (no longer manufactured or distributed), BIOFIRE 2.0 system, and BIOFIRE Torch system.
5. CSF specimens should not be centrifuged, diluted, or otherwise processed prior to testing.
6. This test is not intended for use with CSF collected from indwelling medical devices (e.g., CSF shunts).
7. The performance of this test has not been established for CSF specimens from patients without signs and/or symptoms of meningitis and/or encephalitis.
8. The performance of the BIOFIRE ME Panel has not been specifically evaluated for CSF specimens collected from individuals on antimicrobial treatment.
9. The performance of this test has not been established for monitoring treatment of infection with any of the panel organisms.
10. The performance of this test has not been specifically evaluated for CSF specimens collected from immunocompromised individuals.
11. Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for HSV-1, HSV-2, Human parechovirus, VZV, HHV-6 and *C. neoformans/gattii* were also established with retrospective clinical specimens.
12. Due to the small number of positive prospective and retrospective specimens for certain organisms, performance characteristics for *Escherichia coli*, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae*, Cytomegalovirus, and Human parechovirus were established primarily using contrived clinical specimens.
13. Only *E. coli* strains possessing the K1 capsular antigen will be detected by the BIOFIRE ME Panel. All other *E. coli* strains/serotypes will not be detected.
14. Only encapsulated strains of *N. meningitidis* will be detected by the BIOFIRE ME Panel. Unencapsulated *N. meningitidis* will not be detected.
15. Positive and negative predictive values are highly dependent on prevalence. False positive results are more likely for low prevalence analytes.
16. Patients with a suspicion of cryptococcal meningitis and a negative cryptococcal PCR result, such as by the BIOFIRE ME Panel, should be tested for cryptococcal antigen (CrAg).
17. A negative BIOFIRE ME Panel result does not exclude the possibility of CNS infection and should not be used as the sole basis for diagnosis, treatment, or other management decisions. Negative test results can be observed for several reasons, including CNS infections caused by an organism that is not detected by the BIOFIRE ME Panel.
18. A positive BIOFIRE ME Panel result (detection of organism nucleic acid) does not imply that the corresponding organisms are infectious or the causative agents for clinical symptoms. Viral, bacterial, and yeast nucleic acid may persist *in vivo* independently of organism viability and/or active infection.

19. Viral shedding into the CSF often occurs in cases of zoster (shingles; caused by reactivation of VZV). Detection of VZV in CSF may not indicate the cause of CNS disease in these cases.

20. Herpesviruses (CMV, HHV-6, HSV-1, HSV-2, and VZV) can exist in latent forms that may be reactivated during infection by other pathogens, including agents not detected by the BIOFIRE ME Panel that may cause meningitis/encephalitis (e.g., *Mycobacterium tuberculosis* or HIV). HHV-6 can be chromosomally integrated into somatic cells (ciHHV-6) and into the germ cell line (iciHHV-6) allowing for persistent and sporadic reactivation in confirmed carriers or someone related to a confirmed carrier. When detected by the BIOFIRE ME Panel, herpesvirus results should be considered as the likely cause of meningitis/encephalitis only in appropriate clinical context and following expert consultation.

21. Failure to observe proper procedures for specimen collection, handling, transport, storage, or testing can affect detection of organism nucleic acids and lead to incorrect (false positive or false negative) results.

22. There are multiple potential sources or causes of false positive and/or false negative results. It is important that all BIOFIRE ME Panel test results be interpreted in conjunction with other clinical, epidemiological, or laboratory information.

23. Organism and amplicon contamination may produce erroneous (false positive) results for this test. Particular attention should be given to the Laboratory Precautions noted under the Warnings and Precautions section.

24. False positive results can be caused by contamination of specimens or the test with organisms shed by otherwise healthy individuals. *S. pneumoniae* and *H. influenzae* can be shed from the respiratory tract of healthy individuals. HSV-1 may also be shed from individuals with active or recurrent cold sores. To minimize the risk of false positive results due to contamination, caution should be exercised during specimen collection and testing and particular attention should be given to the Laboratory Precautions noted under the Warnings and Precautions section.

25. Erroneous (false positive) results can be caused by non-specific amplification or cross-reactivity. Organisms with a known risk for cross-reactivity with a BIOFIRE ME Panel assay are indicated in the Analytical Specificity section. Cross-reactivity with additional organisms or organism variants that were not included in the performance evaluation is also possible.

26. The BIOFIRE ME Panel Enterovirus assay can cross-react with human rhinoviruses. Human rhinoviruses are genetically very similar to enteroviruses and can be shed from the respiratory tract of healthy individuals or those with signs and symptoms of a respiratory tract infection. However, rhinoviruses are rarely present in human cerebrospinal fluid and are not a recognized cause of meningitis. Caution should be exercised during specimen collection and testing to prevent contamination with rhinovirus.

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

28. There is a risk of false negative results due to errors in specimen collection, transport, storage or testing, including sample mix-up.

29. False negative results may occur when the concentration of organism(s) in the specimen is below the device limit of detection. Antimicrobial therapy may affect (reduce) organism levels in the specimen.

30. There is a risk of false negative results due to sequence variants, deletions, or rearrangements in the gene targets of the assay. Sequence variants that are known to affect detection of specific BIOFIRE ME Panel analytes (e.g. HSV-1 strain 95/1906) are indicated in the Analytical Reactivity section. An impact on reactivity and detection may also occur with new variants that were not included in the performance evaluation and/or identified from sequence database searches. The identification and/or emergence of new sequence variants that may impact detection is monitored routinely through a post-market surveillance program.

31. There is a risk of erroneous results due to interference or inhibition from substances or nucleic acids in the specimen. The substances and concentrations evaluated for interference effects are indicated in the Interference section. Interference from substances/nucleic acids or concentrations other than those described in the Interference section is possible.

EXPECTED VALUES

In the prospective clinical evaluation of the BIOFIRE ME Panel, 1560 eligible specimens (CSF collected via lumbar puncture) were collected and tested at 11 study sites across the United States over approximately eight months (February – September 2014). The number and percentage of positive results as determined by the BIOFIRE ME Panel, stratified by age group, are presented in the following table. Overall, the BIOFIRE ME Panel detected at least one organism in a total of 136 prospective specimens (8.7% positivity rate), with a total of 141 analyte detections (co-detections were observed in five specimens; see Table 6).

Table 4. Expected Values (as determined by the BIOFIRE ME Panel) Summary by Age Group for the Prospective Clinical Evaluation (February through September 2014)

BIOFIRE ME Panel Result	*Overall (n=1560)*	< 2 mo. (n=299)	2-23 mo. (n=143)	2-17 years (n=197)	18-34 years (n=224)	35-64 years (n=522)	65+ years (n=175)
Bacteria							
E. coli K1	*3 (0.2%)*	0 (0%)	1 (0.7%)	0 (0%)	0 (0%)	2 (0.4%)	0 (0%)
H. influenzae	*2 (0.1%)*	0 (0%)	1 (0.7%)	0 (0%)	0 (0%)	1 (0.2%)	0 (0%)
L. monocytogenes	*0 (0.0%)*	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
N. meningitidis	*0 (0.0%)*	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
S. agalactiae	*1 (0.1%)*	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.6%)
S. pneumoniae	*16 (1.0%)*	2 (0.7%)	2 (1.4%)	2 (1%)	3 (1.3%)	4 (0.8%)	3 (1.7%)
Viruses							
CMV	*6 (0.4%)*	4 (1.3%)	0 (0%)	1 (0.5%)	1 (0.4%)	0 (0%)	0 (0%)
EV	*51 (3.3%)*	31 (10.4%)	5 (3.5%)	11 (5.6%)	4 (1.8%)	0 (0%)	0 (0%)
HSV-1	*4 (0.3%)*	0 (0%)	2 (1.4%)	0 (0%)	0 (0%)	2 (0.4%)	0 (0%)
HSV-2	*12 (0.8%)*	0 (0%)	0 (0%)	0 (0%)	1 (0.4%)	8 (1.5%)	3 (1.7%)
HHV-6	*22 (1.4%)*	9 (3%)	7 (4.9%)	2 (1%)	3 (1.3%)	1 (0.2%)	0 (0%)
HPeV	*12 (0.8%)*	12 (4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
VZV	*7 (0.4%)*	0 (0%)	0 (0%)	0 (0%)	3 (1.3%)	3 (0.6%)	1 (0.6%)
Yeast							
C. neoformans/gattii	*5 (0.3%)*	1 (0.3%)	0 (0%)	0 (0%)	1 (0.4%)	2 (0.4%)	1 (0.6%)

Table 5. BIOFIRE ME Panel Positivity Rate In the Prospective Clinical Evaluation; Overall and By Age Group

Overall (n=1560)	
Negatives	1424 (91.3%)
Positives	136 (8.7%)
Single Detections	131 (8.4%)
Co-Detections	5 (0.3%)
Positivity by Age Group	
< 2 mo. (n=299)	58 (19.4%)
2-23 mo. (n=143)	17 (11.9%)
2-17 years (n=197)	15 (7.6%)
18-34 years (n=224)	15 (6.7%)
35-64 years (n=522)	23 (4.4%)
65+ years (n=175)	8 (4.6%)

In the prospective clinical evaluation, the BIOFIRE ME Panel reported a total of 5 specimens with multiple analytes detected (i.e., mixed infections). This represents 3.7% (5/136) of positive specimens and 0.3% of all specimens tested (5/1560). The expected values for each BIOFIRE ME Panel result in co-detections are presented in the following table.

Table 6. Expected Values for Analytes in Co-detections (as determined by the BIOFIRE ME Panel) in the Prospective Clinical Evaluation (February through September 2014)

Analyte	Prevalence in Co-Detections (n=5)	
Bacteria		
E. coli K1	0	0%
H. influenzae	0	0%
L. monocytogenes	0	0%
N. meningitidis	0	0%
S. agalactiae	1	20%
S. pneumoniae	2	40%
Viruses		
CMV	1	20%
EV	1	20%
HSV-1	1	20%
HSV-2	1	20%
HHV-6	1	20%
HPeV	1	20%
VZV	1	20%
Yeast		
C. neoformans/gattii	0	0%

PERFORMANCE CHARACTERISTICS

Clinical and non-clinical studies have established that the performance characteristics of BIOFIRE ME Panel, including LoD (see Limit of Detection section below), positive percent agreement and negative percent agreement, and reproducibility, are equivalent on BIOFIRE FILMARRAY System and BIOFIRE 2.0 systems. Non-clinical studies also demonstrate similar performance characteristics on BIOFIRE Torch systems.

NOTE: BIOFIRE Torch Modules are BIOFIRE 2.0 instruments that have been re-configured into a stacked system for higher-throughput in a smaller workspace.

Clinical Performance

The clinical performance of the BIOFIRE ME Panel was established during a multi-center study conducted at 11 geographically distinct U.S. study sites between February and September 2014. Specimens enrolled between February and June were collected and immediately frozen for later testing at the source laboratory. A total of 1643 prospective CSF specimens were acquired for the clinical study; 83 of these were excluded. The most common reason for specimen exclusion was that the specimen was found to not meet the inclusion criteria after the specimen had been enrolled. The final data set consisted of 1560 specimens, of which 545 (35%) had been previously frozen before testing. Table 7 provides a summary of demographic information for the 1560 specimens included in the prospective study.

Table 7. Demographic Summary for Prospective BIOFIRE ME Panel Clinical Evaluation

Prospective Study Specimens (%)	
Fresh	1015 (65%)
Frozen	545 (35%)
Total Specimens	1560
Sex	**Number of Specimens (%)**
Male	797 (51%)
Female	763 (49%)
Age Group	**Number of Specimens (%)**
< 2 mo.	299 (19%)
2-23 mo.	143 (9%)
2-17 years	197 (13%)
18-34 years	224 (14%)
35-64 years	522 (33%)
65+ years	175 (11%)
Status	**Number of Specimens (%)**
Outpatient	112 (7%)
Hospitalized	920 (59%)
Emergency	528 (34%)

The performance of the BIOFIRE ME Panel was evaluated by comparing the BIOFIRE ME Panel test result for each member of the panel with the appropriate comparator/reference methods shown in the table below.

Table 8. Comparator Methods for BIOFIRE ME Panel Clinical Evaluation

FilmArray Analyte	Comparator Method	Comparator Test Location
E. coli K1	CSF bacterial culture	Source Laboratory
H. influenzae		
L. monocytogenes		
N. meningitidis		
S. agalactiae		
S. pneumoniae		
CMV	Two PCR assays with bi-directional sequencing[a]	BioFire Laboratory
EV		
HSV-1		
HSV-2		
HHV-6		
HPeV		
VZV		
C. neoformans/gattii		

[a] All assays targeted different nucleic acid sequences than those identified by the BIOFIRE ME Panel.

A total of 1560 specimens were evaluated in this study. Clinical sensitivity or positive percent agreement (PPA) was calculated as 100% x (TP / (TP + FN)). True positive (TP) indicates that both the BIOFIRE ME Panel and reference/comparator method had a positive result for the specific analyte, and false negative (FN) indicates that the BIOFIRE ME Panel result was negative while the comparator result was positive. Specificity or negative percent agreement (NPA) was calculated as 100% x (TN / (TN + FP)). True negative (TN) indicates that both the BIOFIRE ME Panel and the reference/comparator method had negative results, and a false positive (FP) indicates that the BIOFIRE ME Panel result was positive but the comparator result was negative. The two-sided 95% confidence intervals were calculated.

Table 9. BIOFIRE ME Panel Prospective Clinical Performance Summary[a]

Analyte		Sensitivity (compared to culture)			Specificity (compared to culture)		
		TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Bacteria							
E. coli K1	Fresh	1/1	100	-	1014/1014	100	99.6-100
	Frozen	1/1	100	-	543/544	99.8	99.0-100
	Overall	***2/2***	***100***	***34.2-100***	***1557/1558[b,c]***	***99.9***	***99.6-100***
H. influenzae	Fresh	1/1	100	-	1013/1014	99.9	99.4-100
	Frozen	0/0	-	-	545/545	100	99.3-100
	Overall	***1/1***	***100***	***-***	***1558/1559[d]***	***99.9***	***99.6-100***
L. monocytogenes	Fresh	0/0	-	-	1015/1015	100	99.6-100
	Frozen	0/0	-	-	545/545	100	99.3-100
	Overall	***0/0***	***-***	***-***	***1560/1560***	***100***	***99.8-100***
N. meningitidis	Fresh	0/0	-	-	1015/1015	100	99.6-100
	Frozen	0/0	-	-	545/545	100	99.3-100
	Overall	***0/0***	***-***	***-***	***1560/1560***	***100***	***99.8-100***
S. agalactiae	Fresh	0/1	0.0	-	1013/1014	99.9	99.4-100
	Frozen	0/0	-	-	545/545	100	99.3-100
	Overall	***0/1[e]***	***0.0***	***-***	***1558/1559[e]***	***99.9***	***99.6-100***

S. pneumoniae	Fresh	2/2	100	34.2-100	1008/1013	99.5	98.8-99.8
	Frozen	2/2	100	34.2-100	536/543	98.7	97.4-99.4
	Overall	***4/4***	***100***	***51.0-100***	***1544/1556[f]***	***99.2***	***98.7-99.6***
Analyte		**Positive Percent Agreement (compared to PCR with bi-directional sequencing)**			**Negative Percent Agreement (compared to PCR with bi-directional sequencing)**		
		TP/(TP + FN)	**%**	**95% CI**	**TN/(TN + FP)**	**%**	**95% CI**
Viruses							
CMV	Fresh	2/2	100	34.2-100	1010/1013	99.7	99.1-99.9
	Frozen	1/1	100	20.7-100	544/544	100	99.3-100
	Overall	***3/3***	***100***	***43.9-100***	***1554/1557[g]***	***99.8***	***99.4-99.9***
EV	Fresh	43/44	97.7	88.2-99.6	965/971	99.4	98.7-99.7
	Frozen	1/2	50.0	-	542/543	99.8	99.0-100
	Overall	***44/46[h]***	***95.7***	***85.5-98.8***	***1507/1514[h]***	***99.5***	***99.0-99.8***
HSV-1	Fresh	1/1	100	-	1013/1014	99.9	99.4-100
	Frozen	1/1	100	-	543/544	99.8	99.0-100
	Overall	***2/2***	***100***	***34.2-100***	***1556/1558[i]***	***99.9***	***99.5-100***
HSV-2	Fresh	6/6	100	61.0-100	1008/1009	99.9	99.4-100
	Frozen	4/4	100	51.0-100	540/541	99.8	99.0-100
	Overall	***10/10***	***100***	***72.2-100***	***1548/1550[j]***	***99.9***	***99.5-100***
HHV-6	Fresh	13/15	86.7	62.1-96.3	997/1000	99.7	99.1-99.9
	Frozen	5/6	83.3	43.6-97.0	535/536	99.8	99.0-100
	Overall	***18/21[k]***	***85.7***	***65.4-95.0***	***1532/1536[k]***	***99.7***	***99.3-99.9***
HPeV	Fresh	9/9	100	70.1-100	1003/1006	99.7	99.1-99.9
	Frozen	0/0	-	-	545/545	100	99.3-100
	Overall	***9/9***	***100***	***70.1-100***	***1548/1551[l]***	***99.8***	***99.4-99.9***
VZV	Fresh	3/3	100	43.9-100	1010/1012	99.8	99.3-99.9
	Frozen	1/1	100	-	543/544	99.8	99.0-100
	Overall	***4/4***	***100***	***51.0-100***	***1553/1556[m]***	***99.8***	***99.4-99.9***
Yeast							
C. neoformans/gattii	Fresh	0/0	-	-	1015/1015	100	99.6-100
	Frozen	1/1	100	-	540/544	99.3	98.1-99.7
	Overall	***1/1***	***100***	***-***	***1555/1559[n]***	***99.7***	***99.3-99.9***

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

[b] The FP specimen was negative for *E. coli* K1 when tested using an independent PCR assay (targeting a nucleic acid region distinct from that identified by the BIOFIRE ME Panel). Meningitis was clinically excluded in this patient.

[c] An additional infant presented with CSF pleocytosis (WBC 3738) and *E. coli* bacteremia. CSF cultures and BIOFIRE ME Panel were negative, but no information regarding pre-treatment with antibiotics was available, and the patient was clinically diagnosed with meningitis.

[d] *H. influenzae* was detected in the single FP specimen using an independent PCR assay and was also observed via Gram stain; the subject from whom this specimen was collected received a physician diagnosis of gram-negative bacterial meningitis.

[e] The laboratory reported that *S. agalactiae* was present at a very low level (two colonies) for the FN specimen. The FP specimen was negative for *S. agalactiae* when tested using an independent PCR assay.

[f] *S. pneumoniae* was detected in 5/12 FP specimens using an independent PCR assay; additional information regarding seven unconfirmed FP specimens is detailed below in Table 10.

[g] CMV was detected in 1/3 FP specimens using an independent PCR assay.

[h] EV was detected in 2/2 FN specimens using an independent PCR assay; one specimen was positive upon FilmArray ME retest. EV was detected in 5/7 FP specimens using an independent PCR assay.

[i] Both FP specimens were negative for HSV-1 when tested using an independent PCR assay.

[j] HSV-2 was detected in 1/2 FP specimens using an independent PCR assay; the subject from whom this specimen was collected received a physician diagnosis of HSV meningitis.

[k] HHV-6 was detected in 2/3 FN and 1/4 FP specimens using an independent PCR assay.

[l] HPeV was detected in 1/3 FP specimens using an independent PCR assay; the subject from whom this specimen was collected received a physician diagnosis of HPeV meningitis. Both of the subjects from whom the remaining two specimens were collected received a diagnosis of HPeV infection following detection of HPeV in the blood.
[m] VZV was detected in 1/3 FP specimens using an independent PCR assay; the subject from whom this specimen was collected received a physician diagnosis of herpes zoster. Of the remaining two specimens with FP results, one was collected from a subject who was diagnosed with herpes zoster oticus.
[n] *C. neoformans/gattii* was detected in 2/4 FP specimens using a commercially available antigen test. One FP specimen was positive by standard culture. Additional information regarding BIOFIRE ME Panel performance with respect to cryptococcal antigen testing is detailed below.

Of 12 false positive *S. pneumoniae*, seven could not be confirmed using an independent PCR assay. A review of subject medical data was conducted for the subjects from whom these specimens were collected and is summarized below in Table 10. None of the subjects had evidence of bacterial meningitis/encephalitis. The cause of these false positives was not determined.

Table 10. Clinical Characteristics of Subjects with Unconfirmed False Positive *S. pneumoniae* Results

Subject age	CSF WBC	BIOFIRE Result	Comparator Culture/ Investigation PCR[a]	Diagnosis Reported in Medical Record
<2 mo	3	Pos	Neg/Neg	Infection, non-CNS (*S. agalactiae* urine culture)
65+	2	Pos	Neg/Neg	*Unable to obtain*
2-17	0	Pos	Neg/Neg	Infection, non-CNS (folliculitis)
<2 mo	3	Pos	Neg/Neg	Infection, non-CNS (Parainfluenza virus)
18-34	1	Pos	Neg/Neg	CNS disease, non-infectious (epilepsy)
35-64	1	Pos	Neg/Neg	Infection, non-CNS (Hep B), multiple myeloma
18-34	1	Pos	Neg/Neg	Infection, non-CNS (Bells' Palsy)

[a] This PCR is the same as that described in footnote f of Table 9.

The comparator method use to evaluate BIOFIRE ME Panel *C. neoformans/gattii* performance was PCR with bi-directional sequencing. BIOFIRE ME Panel performance for detection of *Cryptococcus* was also calculated in comparison to specific testing for *Cryptococcus* that was performed by the laboratory based upon clinician test requests for a subset of subjects. For data that were available, BIOFIRE ME Panel performance is shown in Table 11 relative to cryptococcal antigen testing (N=196), standard culture (N=1560), and fungal culture (N=23). Notably, seven out of eight CrAg-positive specimens were discordant with BIOFIRE ME Panel results. All seven of these specimens were negative for *Cryptococcus* when tested with both PCR comparator assays. Medical chart review indicated that each subject was on antifungal therapy for treatment of cryptococcal meningitis or cryptococcosis at the time of specimen collection and/or had prior history of *Cryptococcus* infection. Therefore, positive antigen results for these patients in the absence of PCR and culture-based organism detection are likely due to antigen persistence rather than the presence of live organism.

Table 11. BIOFIRE ME Panel C. *neoformans/gattii* assay performance relative to other comparator methods

Cryptococcus test comparator method	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Cryptococcal Antigen	1/8[a]	12.5	2.2-47.1	187/188[b]	99.5	97.0-99.5
Standard Culture	2/3[c]	66.7	20.8-93.9	1554/1557[d]	99.8	99.4-99.9
Fungal Culture	0/0	-	-	22/23[e]	95.7	79.0-99.2

[a] Seven specimens were positive by CrAg testing performed at the clinical site, but were negative by standard of care culture, BIOFIRE ME Panel, and two comparator assays. All seven subjects from whom these specimens were collected received antifungal therapy prior to LP and/or had prior history of *Cryptococcus* infection. The eighth specimen was positive by CrAg, BIOFIRE ME Panel, and standard culture.
[b] *Cryptococcus* was detected in the single FP specimen using a CrAg testing kit at BioFire.
[c] The single FN specimen was also positive by standard culture, but negative by the BIOFIRE ME Panel and two comparator assays. The laboratory reported that only one colony was recovered.
[d] *Cryptococcus* was detected in 1/3 FP specimens using CrAg testing kits at BioFire (this is the same FP described in footnote b).
[e] The single FP specimen was negative by standard of care culture, CrAg testing performed at the clinical site, and negative by two comparator assays, but was positive by CrAg testing performed at BioFire (this is the same FP described in footnote b).

The BIOFIRE ME Panel reported a total of 5 specimens with discernible multiple organism detections (0.3% of all specimens, 5/1560; and 3.7% of positive specimens, 5/136. Each multi-analyte detection contained two organisms, at least one of which was not detected by the reference/comparator method (i.e., each specimen contained at least one false positive result).

Table 12. Co-detection Combinations as Determined by the BIOFIRE ME Panel

Co-detection Combination	Number of Specimens	Discrepant Analytes (detected by BIOFIRE only)
CMV + *S. pneumoniae*	1	CMV
EV + HPeV	1	EV
HSV-1 + HHV-6	1	HSV-1
HSV-2 + *S. agalactiae*	1	*S. agalactiae*
S. pneumoniae + VZV	1	*S. pneumoniae*, VZV

The overall BIOFIRE ME Panel test success rate on the initial test of these specimens was 98.9% (1560/1577); 17 tests were unsuccessful (11 due to incomplete tests and six due to control failures). No pouch leaks were observed.

Testing of Preselected Archived Specimens

Several analytes were either not encountered or had a low prevalence in the prospective clinical study. To supplement the results of the prospective clinical study, an evaluation of 235 preselected archived specimens (of which 25 were negative) was performed. These specimens were archived clinical specimens that were selected because they had previously tested positive for one of the following analytes: *Cryptococcus*, CMV, *E. coli*, *H. influenzae*, HSV-1, HSV-2, HHV-6, HPeV, *L. monocytogenes*, *N. meningitidis*, *S. agalactiae*, *S. pneumoniae*, and VZV; or had been negative in previous laboratory testing. Prior to testing with the BIOFIRE ME Panel, the presence (or absence) of the expected analytes was verified in each specimen using a confirmatory molecular test (e.g. PCR with bi-directional sequencing) Out of the 210 positives, the historical result was confirmed by the comparator method for 150 (150/210; 71.4%); only confirmed analytes were used in calculations of PPA, but all specimens were used for NPA analyses as presented in Table 15.

The specimens were organized into “test panels” and randomized such that the users performing the BIOFIRE ME Panel testing were blinded as to the expected test result. A summary of the available demographic information of the tested samples is provided in Table 13 and the results of the BIOFIRE ME Panel testing are presented in Table 14.

Table 13. Demographic Summary

Preselected Archived Specimens	
Total Specimens	235
Sex	**Number of Specimens (%)**
Male	70 (30%)
Female	90 (38%)
Unknown	75 (32%)
Age Group	**Number of Specimens (%)**
<2 mo	5 (2%)
2-23 mo	19 (8%)
2-17 yrs.	19 (8%)
18-34 yrs.	33 (14%)
35-64 yrs.	65 (28%)
65+ yrs.	26 (11%)
Unknown	68 (29%)

Table 14. BIOFIRE ME Panel Archived Specimen Performance Data Summary

Analyte	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Bacteria						
E. coli K1	2/2	100	34.2-100	35/35	100	90.1-100
H. influenzae	3/3	100	43.9-100	39/39	100	91-100
L. monocytogenes	1/1	100	-	41/41	100	91.4-100
N. meningitidis	7/7	100	64.6-100	34/34	100	89.8-100
S. agalactiae	2/2	100	34.2-100	40/40	100	91.2-100
S. pneumoniae	17/17	100	81.6-100	21/21	100	84.5-100
Viruses						
CMV	7/8	87.5	52.9-97.8	181/181	100	97.9-100
HSV-1	16/16	100	80.6-100	156/157	99.4	96.5-99.9
HSV-2	33/34	97.1	85.1-99.5	136/136	100	97.3-100
HHV-6	12/16[a]	75.0	50.5-89.8	168/168	100	97.8-100
HPeV	2/3	66.7	20.8-93.9	187/187	100	98.0-100
VZV	22/22	100	85.1-100	162/164	98.8	95.7-99.7
Yeast						
C. neoformans/gattii	19/19[b]	100	83.2-100	171/171	100	97.8-100

[a] Two specimens were sequenced and identified as HHV-6A while 14 were HHV-6B. Of the four FilmArray FN specimens, one was sequenced and identified as HHV-6A and the remaining three FN specimens were identified as HHV-6B. The resulting PPA was 50% (1/2); 95% CI 9.5 – 90.5% and 79% (11/14); 95% CI 52.4 – 92.4% for HHV-6A and HHV-6B, respectively.
[b] One specimen was sequenced and identified as *C. gattii* and 18 were *C. neoformans*.

Testing of Contrived Specimens

Several analytes, such as *Haemophilus influenzae*, were not detected in sufficient numbers in both prospective and archived testing efforts to demonstrate system performance. To supplement the prospective and archived data, an evaluation of contrived specimens was performed. Surrogate specimens were prepared using residual specimens that had previously tested negative for all BIOFIRE ME Panel analytes by BIOFIRE and comparator methods. For each analyte, at least 25 specimens were spiked at 2 × LoD and the remaining were spiked at four additional concentrations spanning the clinically relevant range using at least five different quantified strains for each organism. Specimens were prepared and randomized along with negative (unspiked) specimens such that the analyte status of each contrived specimen was blinded to the users analyzing the specimens. Contrived specimens were frozen, then distributed to prospective clinical study sites for testing. The results of the BIOFIRE testing are presented in Table 15.

Table 15. BIOFIRE ME Panel Performance Using Contrived Specimens

Analyte	PPA			NPA		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
E. coli K1	47/49[a]	95.9	86.3-98.9	245/245	100	98.5-100
H. influenzae	50/50	100	92.9-100	243/244	99.6	97.7-99.9
L. monocytogenes	50/50	100	92.9-100	244/244	100	98.5-100
N. meningitidis	75/75	100	95.1-100	219/219	100	98.3-100
S. agalactiae	48/50[b]	96.0	86.5-98.9	244/244	100	98.5-100
CMV	47/49[c]	95.9	86.3-98.9	245/245	100	98.5-100
HHV-6	50/50	100	92.9-100	243/244	99.6	97.7-99.9

Analyte	PPA			NPA		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
HPeV	50/50	100	92.9-100	244/244	100	98.5-100

[a] One *E coli* K1 false negative was observed at 2 × LoD and one *E coli* K1 false negative was observed at 0.2 × LoD.
[b] Both *S. agalactiae* false negatives were observed at 0.2 × LoD.
[c] Both CMV false negatives were observed at 0.2 × LoD.

BIOFIRE 2.0 Clinical Comparison

To demonstrate that performance of the BIOFIRE ME Panel when used with BIOFIRE 2.0 is equivalent to BIOFIRE FILMARRAY System, a combination of residual, de-identified CSF specimens and contrived CSF specimens covering all 14 analytes on the BIOFIRE ME Panel were evaluated. A total of 149 specimens were tested consisting of 21 clinical specimens and 128 contrived specimens. Each analyte was represented a minimum of five times in the specimen set. All specimens were evaluated on both systems. The results of the testing are presented in Table 16 (BIOFIRE 2.0 results are represented in the numerator and BIOFIRE FILMARRAY System results are the denominator).

Table 16. Clinical Performance Comparison Summary of BIOFIRE ME Panel When Tested on BIOFIRE 2.0 (FA2.0) and BIOFIRE FILMARRAY (FA)

Analyte		Positive Percent Agreement			Negative Percent Agreement		
		FA2.0/FA	%	95% CI	FA2.0/FA	%	95% CI
Bacteria							
E. coli K1	Clinical	0/0	-	-	21/21	100%	84.5-100%
	Contrived	5/6	83.3%	43.7-97.0%	122/122	100%	97.0-100%
	Overall	5/6	83.3%	43.7-97.0%	143/143	100%	97.4-100%
H. influenzae	Clinical	0/0	-	-	21/21	100%	84.5-100%
	Contrived	10/10	100%	72.3-100%	118/118	100%	96.9-100%
	Overall	10/10	100%	72.3-100%	139/139	100%	97.3-100%
L. monocytogenes	Clinical	0/0	-	-	21/21	100%	84.5-100%
	Contrived	5/5	100%	56.6-100%	123/123	100%	97.0-100%
	Overall	5/5	100%	56.6-100%	144/144	100%	97.4-100%
N. meningitidis	Clinical	0/0	-	-	21/21	100%	84.5-100%
	Contrived	11/11	100%	74.1-100%	117/117	100%	96.8-100%
	Overall	11/11	100%	74.1-100%	138/138	100%	97.3-100%
S. agalactiae	Clinical	1/1	100%	20.7-100%	20/20	100%	83.9-100%
	Contrived	5/5	100%	56.6-100%	123/123	100%	97.0-100%
	Overall	6/6	100%	61.0-100%	143/143	100%	97.4-100%
S. pneumoniae	Clinical	1/1	100%	20.7-100%	20/20	100%	83.9-100%
	Contrived	6/7	85.7%	48.7-97.4%	120/121	99.2%	95.5-99.9%
	Overall	7/8	87.5%	52.9-97.8%	140/141	99.3%	96.1-99.9%
Viruses							
CMV	Clinical	1/1	100%	20.7-100%	20/20	100%	83.9-100%
	Contrived	5/5	100%	56.6-100%	123/123	100%	97.0-100%
	Overall	6/6	100%	61.0-100%	143/143	100%	97.4-100%
EV	Clinical	1/1	100%	20.7-100%	20/20	100%	83.9-100%
	Contrived	11/12	91.7%	64.6-98.5%	114/116	98.3%	93.9-99.5%
	Overall	12/13	92.3%	66.7-98.6%	134/136	98.5%	94.8-99.6%
HSV-1	Clinical	3/3	100%	43.9-100%	18/18	100%	82.4-100%
	Contrived	3/3	100%	43.9-100%	125/125	100%	97.0-100%

Analyte		Positive Percent Agreement			Negative Percent Agreement		
		FA2.0/FA	%	95% CI	FA2.0/FA	%	95% CI
	Overall	6/6	100%	61.0-100%	143/143	100%	97.4-100%
HSV-2	Clinical	2/2	100%	34.2-100%	19/19	100%	83.2-100%
	Contrived	3/3	100%	43.9-100%	125/125	100%	97.0-100%
	Overall	5/5	100%	56.6-100%	144/144	100%	97.4-100%
HHV-6	Clinical	3/3	100%	43.9-100%	18/18	100%	82.4-100%
	Contrived	9/9	100%	70.1-100%	117/119	98.3%	94.1-99.5%
	Overall	12/12	100%	75.8-100%	135/137	98.5%	94.8-99.6%
HPeV	Clinical	0/0	-	-	21/21	100%	84.5-100%
	Contrived	8/8	100%	67.6-100%	120/120	100%	96.9-100%
	Overall	8/8	100%	67.6-100%	141/141	100%	97.4-100%
VZV	Clinical	3/3	100%	43.9-100	18/18	100%	82.4-100%
	Contrived	4/4	100%	51.0-100%	124/124	100%	97.0-100%
	Overall	7/7	100%	64.6-100%	142/142	100%	97.4-100%
Yeast							
C. neoformans/gattii	Clinical	2/2	100%	34.2-100%	19/19	100%	83.2-100%
	Contrived	15/15	100%	79.6-100%	112/113	99.1%	95.2-99.9%
	Overall	17/17	100%	81.6-100%	131/132	99.2%	95.8-99.9%
Overall Agreement		**117/120**	**97.5%**	**92.9-99.2%**	**1960/1966**	**99.7%**	**99.3-99.9%**

The BIOFIRE ME Panel demonstrated 100% concordance for the 21 individual clinical specimens. Additionally, 100% concordance was also observed for nine out of 14 analytes when evaluating contrived specimens. Occasional discrepant results were observed; this was likely due to differential detection resulting from spiking near the LoD or unexpected detection of low-level (sub-LoD) analyte present as background in the clinical matrix that had been originally characterized as negative. Overall PPA for clinical and contrived specimens combined was 97.5% with the lower bound of the two-sided 95% confidence interval (95% CI) at 92.9%, and overall NPA was 99.7% with the lower bound of the two-sided 95% CI at 99.3%

Limit of Detection

The limit of detection (LoD) for BIOFIRE ME Panel analytes was estimated by testing dilutions of contrived samples containing known concentrations of organisms (bacteria, viruses, and yeast). Confirmation of LoD was achieved by testing 20 replicates of a contrived sample containing analytes at their estimated LoD concentration. LoD was confirmed when the organism was detected in at least 19 of the 20 replicates tested (19/20 = 95%). When available, World Health Organization (WHO) International Standards were also included in the LoD confirmation testing.

The confirmed LoD for each BIOFIRE ME Panel analyte tested on the BIOFIRE 2.0 and BIOFIRE Torch systems is listed in Table 17.

NOTE: For bacteria and yeast, LoD concentrations are provided in units of CFU/mL (based on plate enumeration) or cells/mL (estimated based on OD_{600}). CFU/mL is a measure of viable cells and may be an underestimate of the amount of genetic material (nucleic acid) contained in the sample, especially for fastidious or autolytic bacteria (e.g. N. meningitidis and S. pneumoniae).

For most viruses, LoD is provided in units of $TCID_{50}$/mL (TCID = tissue culture infectious dose). $TCID_{50}$/mL is not a direct count of viral particles or nucleic acid, but an indirect representation of viral concentration based on infectivity and cytotoxicity. $TCID_{50}$ measurements can be highly variable and dependent on methodology, technique, and other parameters including the infected cell type, culture media and conditions, cytotoxicity of the virus, etc. It is not appropriate to make determinations on relative sensitivity of detection for different isolates, types, cultures, and/or between different molecular assays based on LoD values measured in $TCID_{50}$/mL.

For some viruses and bacteria, LoD is (also) listed in nucleic acid copies/mL determined by molecular quantification with a quantitative real-time PCR assay (qPCR). The accuracy of qPCR concentrations may be affected by extraction efficiency, standard curve accuracy, assay conditions, inhibitors, and/or sequence. The qPCR quantification has not been compared to a reference material or other quantification methods.

The concentration of WHO International Standards is indicated in International Units (IU)/mL. IU are established in worldwide collaborative studies, as noted in the Instructions for Use provided with the standard.

Table 17. Limit of Detection (LoD) for BIOFIRE ME Panel Analytes

ME Panel Analyte	Species/Isolate Tested	LoD Concentration[a]
BACTERIA		
***E. coli* K1**	*E. coli* K1, strain C5 [Bort]; type O18ac:K1:H7 ATCC 700973	1.0E+03 CFU /mL
H. influenzae	*H. influenzae*, strain AMC 36-A-1 [572] type b, biotype I ATCC 10211	1.0E+03 CFU/mL
L. monocytogenes	*L. monocytogenes*, strain 1071/53, type 4b ATCC 13932	1.0E+03 CFU/mL
N. meningitidis	*N. meningitidis*, strain M-1574 [199/W135] ATCC 43744	1.0E+02 CFU/mL (~ 1.80E+03 copies/mL)
S. agalactiae	*S. agalactiae*, type strain, G19, group B ATCC 13813	1.0E+03 CFU/mL
S. pneumoniae	*S. pneumoniae*, strain SV 1, serotype 1 ATCC 33400	1.0E+02 cells/mL (~1.50E+03 copies/mL)
VIRUSES		
CMV	CMV, AD-169 Zeptometrix 0810003CF	1.0E+02 $TCID_{50}$/mL (4.30E+03 copies/mL or IU/mL[b])
	CMV, Merlin WHO International Standard (NIBSC 09/162)[b]	
EV[c] (Species A-D)	Coxsackievirus A6, species A, strain Gdula ATCC VR-1801	5.0E+01 $TCID_{50}$/mL[c]
	Coxsackievirus A9, species B Zeptometrix 0810017CF	
	Coxsackievirus A17, species C, strain G-12 ATCC VR-1023	
	EV 70, species D, strain J670/71 ATCC VR-836	
HSV-1	HSV-1, strain MacIntyre Zeptometrix 0810005CF[d]	2.5E+02 $TCID_{50}$/mL (1.51E+03 copies/mL[d])
HSV-2	HSV-2, strain MS Zeptometrix 0810006CF	5.0E+01 $TCID_{50}$/mL (1.29E+03 copies/mL)
HHV-6	HHV-6A, strain U1102 NCPV 0003121v	1.0E+04 copies/mL or IU/mL[b]
	HHV-6B, strain HST NCPV 0006111v	
	HHV-6B, strain Z-29 WHO International Standard (NIBSC 15/266)[b]	
HPev	HPeV, type 3 Zeptometrix 0810147CF	5.0E+02 $TCID_{50}$/mL
VZV	VZV, strain Ellen Zeptometrix 0810171CF	1.0E-01 $TCID_{50}$/mL (1.66E+03 copies/mL)

ME Panel Analyte	Species/Isolate Tested	LoD Concentration[a]
YEAST		
C. neoformans/gattii	*C. neoformans* var. *grubii*, type strain, H99 [H99JP, NYSD 1649] ATCC 208821	1.0E+02 CFU/mL
	C. gattii, strain A6MR38, AFLP6C, VGIIc ATCC MYA-4877	

[a] Accuracy and reproducibility of LoD concentrations is dependent on the accuracy and precision of the method(s) used to quantify the test material.
[b] International Standards were detected in ≥95% of replicates tested at the indicated IU/mL concentration. Note that IU/mL may not be equal to genomic copies/mL.
[c] Each enterovirus species/serotype tested was detected in ≥95% of replicates tested at a concentration of 50 $TCID_{50}$/mL or lower (e.g. detection of the Coxsackievirus A9 and Coxsackievirus A17 isolate cultures was ≥95% at 5 $TCID_{50}$/mL). *In silico* sequence analysis predicts equivalent detection of nucleic acids from all Enterovirus species and serotypes (see Analytical Reactivity section).
[d] Note that the HSV-1 molecular panel from Qnostics (HSV1MQP01) is composed of a viral strain (95) with sequence variation under an assay primer that will not be reliably detected at the indicated copies/mL LoD (see Analytical Reactivity section).

Analytical Reactivity (Inclusivity)

The analytical reactivity (inclusivity) of the BIOFIRE ME Panel was evaluated with a collection of isolates that represent the diversity of the BIOFIRE ME Panel analytes. Isolates from different geographical areas were selected to represent relevant and/or common species, serotypes, or other known variants. Each isolate was tested and detected at concentrations near (1-3×) the limit of detection (LoD) or within 10× LoD (see footnotes). *In silico* analysis of sequence data was used to make predictions of assay reactivity for less common strains, serotypes, genotypes, or other variants that were not tested.

A summary of BIOFIRE ME Panel reactivity based on a combination of testing and sequence-based reactivity predictions is provided in Table 18 and all isolates tested are listed in Table 19 -Table 20. Limitations on BIOFIRE ME Panel reactivity are noted if detection was impaired by more than 10× LoD and/or if sequence variants or deletions that are predicted to affect detection near LoD have been identified.

Table 18. Summary of BIOFIRE ME Panel Analytical Reactivity (Inclusivity) Testing and i*n silico* Predictions

BIOFIRE ME Panel Analyte	# of Isolates Tested	Concentration Tested/Detected[a]	Description of Isolates Tested/Detected (see Table 19 and Table 20)	Reactivity Limitations
Bacteria				
E. coli K1	5	1.0E–03 - 3.0E+03 CFU/mL	Multiple isolates of *E. coli* serotype K1	None
H. influenzae	9	1.0E–03 - 3.0E+03 CFU/mL	Multiple isolates of non-typeable and typeable (types a-f) *H. influenzae,* including genospecies *quentini*	No serotype or genospecies-specific limitations on detection identified. However, post-market surveillance activities have identified uncommon, sequence variants that when present in a non-typeable strain, will not be detected near LoD (~100-fold impairment).
L. monocytogenes	6	1.0E–03 - 3.0E+03 CFU/mL	Multiple isolates of the most common serotypes (1/2a, 1/2b, and 4b) of *L. monocytogenes*	None; *in silico* analysis predicts the assay will react with all sequences evaluated (no serotype limitations identified)
N. meningitidis	7	1.0E–02 - 3.0E+02 CFU/mL	Multiple isolates and serotypes of encapsulated *N. meningitidis* (and DNA from a strain with a variant *ctrA* gene)	None; *in silico* analysis predicts the assay will react with all sequences/serotypes evaluated or tested (29E, A, B, C, D, H, W, W135, X, Y, and Z)
S. agalactiae	5	1.0E–03 - 3.0E+03 CFU/mL	Multiple isolates of *S. agalactiae* (Group B *Streptococcus*)	None; *in silico* analysis predicts the assay will react with all sequences/serotypes evaluated or tested (Ia, Ib, II, III, V, VIII, and unknown)
S. pneumoniae	7	1.0E–02 - 3.0E+02 cells/mL	Multiple isolates and serotypes of *S. pneumoniae*	None; *in silico* analysis predicts the assay will react with all sequences/serotypes evaluated or tested (1-7F,10, 11A, 12, 14, 19, 19A, 19F, 23, 23F, 29, 35, non-typeable and unknown)

BIOFIRE ME Panel Analyte	# of Isolates Tested	Concentration Tested/Detected[a]	Description of Isolates Tested/Detected (see Table 19 and Table 20)	Reactivity Limitations
Viruses				
CMV	5	4.3E+03 -1.3E+04 copies/mL	Multiple isolates of CMV	None
EV	25	5.0E–00 - 1.5E+02 $TCID_{50}$/mL	Representative isolates from all species (A-D) and several serotypes of human Enterovirus, Coxsackievirus, and Echovirus	No species or serotype-specific limitations on detection identified. *In silico* analysis predicts the assay will react with >98% of sequences evaluated[b], representing >100 serotypes within species A-D (enteroviruses, coxsackieviruses, echoviruses and polioviruses).
HSV-1	6	1.5E–03 - 4.5E+03 copies/mL	Multiple isolates of HSV-1	Post-market surveillance activities have identified two variant sequences that are predicted to impact detection ~10-fold or more[c]
HSV-2	5	1.3E–03 - 3.9E+03 copies/mL	Multiple isolates of HSV-2	None
HHV-6	4	1.0E–04 - 3.0E+04 copies/mL	A and B variants of HHV-6	None
HPeV	6	5.0E–02 - 1.5E+03 $TCID_{50}$/mL[d]	Serotypes 1-6 of HPeV	None; *In silico* analysis predicts the assay will react with sequences representing HPeV serotype– 1 - 8.
VZV	5	1.7E–03 - 5.0E+03 copies/mL	Multiple isolates of VZV	None[e]
Yeast				
C. neoformans/gattii	10	1.0E–02 - 3.0E+02 CFU/mL	Multiple isolates of various serotypes, genotypes, and variants of *Cryptococcus neoformans* and *Cryptococcus gattii*	None

[a] All isolates were detected at a 1-3× LoD or lower concentration, unless otherwise noted.

[b] Enterovirus sequences that may not be detected have one or more mismatched bases in a critical position of one or more assay primers. All of the mismatched sequences evaluated correspond to a minority of annotated sequences within a serotype designation and therefore represent a variant sequence-specific reactivity limitation rather than a serotype-specific reactivity limitation.

[c] Detection of a HSV-1 variant (95/1906; represented by Qnostics HSV1MQP01) with a mismatch to a HSV-1 assay primer is impaired approximately 10-fold (see Table 20). Another HSV-1 variant with a different mismatch to a HSV-1 assay primer was not tested but detection is predicted to be impaired 100-fold or more.

[d] HPeV Serotype 5 was detected at 5.0E+03 $TCID_{50}$/mL (10x LoD), but no limitations on detection of serotype 5 are predicted by sequence analysis.

[e] Based on sequence analysis, the VZV assay is also predicted to detect the Oka strain used in multiple VZV vaccines (monovalent VZV vaccines and VZV vaccine combined with measles-mumps-rubella (MMR)).

Table 19. Bacterial Isolates Tested and Detected by the BIOFIRE ME Panel[a]

Isolate/Source ID	Strain/Serotype Information	Isolate/Source ID	Strain/Serotype Information
***E. coli* K1**		***S. agalactiae***	
ATCC 700973	Serotype O18ac:**K1**:H7	ATCC 13813	Serotype Ia/c
BEI NR-17666	Serotype O2:**K1**:H4	ATCC 12403	Serotype III
BEI NR-17674	Serotype O16:**K1**:H-	ATCC BAA-611	Serotype V
NCTC 9007	Serotype O9:**K1**:H-	Clinical isolate (2010)	Unknown Serotype
NCTC 9045	Serotype O45:**K1**:H10	NCTC 8017	Unknown Serotype
H. influenzae		***N. meningitidis***	
ATCC 51907	Non-typeable [strain Rd [KW20]]	ATCC 43744	Serotype W135
		ATCC 13077	Serotype A
CCUG 36167	Non-typeable, genospecies *quentini*	ATCC 13090	Serotype B
ATCC 9006	Type a [strain AMC 36-A-3]	ATCC 13102	Serotype C
ATCC 31512	Type b [strain Rab]	ATCC 13113	Serotype D
ATCC 10211	Type b [biotype 1]	ATCC 35561	Serotype Y
ATCC 49699	Type c [strain C 9007]	*ctrA* variant DNA	strain with variant *ctrA* gene[84]
ATCC 9008	Type d [strain AMC 36-A-6]		

Isolate/Source ID	Strain/Serotype Information	Isolate/Source ID	Strain/Serotype Information
ATCC 8142	Type e [strain AMC 36-A-7]		
ATCC 700223	Type f [strain GA-1264]	***S. pneumoniae***	
L. monocytogenes		ATCC 33400	Serotype 1
FSL-J2-020	Type 1/2a	ATCC BAA-334	Serotype 4
FSL-C1-056	Type 1/2a	ATCC BAA-341	Serotype 5
FSL-J2-064	Type 1/2b	NCTC 11900	Serotype 11A
Clinical isolate (2009)	Type 1/2b	ATCC 700672	Serotype 14
FSL-J1-110	Type 4b	ATCC 700673	Serotype 19A
ATCC 13932	Type 4b	ATCC 49619	Serotype 19F

[a] All isolates were detected at a 3× LoD or lower concentration.

Table 20. Viral Isolates Tested and Detected by the BIOFIRE ME Panel[a]

Isolate/Source ID	Strain/Serotype Information	Isolate/Source ID	Strain/Serotype Information
CMV		**HSV-1**	
Zeptometrix 0810003CF	strain AD-169	Zeptometrix 0810005CF	Strain MacIntyre
ATCC VR-977	strain Towne	ATCC VR-733	Strain F
ATCC VR-1590	strain Merlin	ATCC VR-260	Strain HF
ATCC VR-807	strain Davis	ATCC VR-1493	Strain KOS
NCPV 0302162v	strain Toledo	ATCC VR-1778	aka ATCC-2011-1
		Qnostics HSV-1 Molecular Q Panel (HSV1MQP01)	Strain 95 (95/1906)[b]
EV		**HSV-2**	
ATCC VR-1801	Coxsackievirus A6	Zeptometrix 0810006CF	strain MS
ATCC VR-168	Coxsackievirus A10	ATCC VR-734	strain G
NCPV 0812071v	Coxsackievirus A16	ATCC VR-1779	aka ATCC-2011-2
NCPV 0812215v	Enterovirus 71	NCPV 0406272v	strain 131596
Zeptometrix 0810017CF	Coxsackievirus A9	NCPV 0104152v	Strain HG52
NCPV 0812078v	Coxsackievirus B1	**HHV-6**	
NCPV 0812142v	Coxsackievirus B2	NCPV 0003121v–6A - strain U1102	
Zeptometrix 0810074CF	Coxsackievirus B3	NCPV 0006111v–6B - strain HST	
Zeptometrix 0810075CF	Coxsackievirus B4	ATCC VR-1480–6B - strain SF	
Zeptometrix 0810019CF	Coxsackievirus B5	Zeptometrix 0810072CF –6B - strain Z29	
ATCC VR-33	Echovirus 3	**HPeV**	
ATCC VR-35	Echovirus 5	Zeptometrix 0810145CF	Serotype 1
Zeptometrix 0810076CF	Echovirus 6	Zeptometrix 0810146CF	Serotype 2
Zeptometrix 0810077CF	Echovirus 9	Zeptometrix 0810147CF	Serotype 3
Zeptometrix 0810023CF	Echovirus 11	Zeptometrix 0810148CF	Serotype 4
ATCC VR-44	Echovirus 14	Zeptometrix 0810149CF	Serotype 5[c]
ATCC VR-46	Echovirus 16	Zeptometrix 0810150CF	Serotype 6
NCPV 0901047v	Echovirus 18	**VZV**	
ATCC VR-49	Echovirus 19	Zeptometrix 0810171CF	Ellen
ATCC VR-1660	Echovirus 30	Zeptometrix 0810172CF	Isolate A
ATCC VR-1023	Coxsackievirus A17	Zeptometrix 0810173CF	Isolate B
ATCC VR-850	Coxsackievirus A21	Zeptometrix 0810168 CF	Strain 275

Isolate/Source ID	Strain/Serotype Information	Isolate/Source ID	Strain/Serotype Information
ATCC VR-583	Coxsackievirus A24	ATCC VR-916	Webster
ATCC VR-836	Enterovirus 70		
Zeptometrix 0810237CF	Enterovirus 68 (aka Rhinovirus 87)		

[a] All isolates were detected at a 3×LoD or lower concentration, unless otherwise noted.
[b] HSV-1 strain 95 (or 95/1906) has a variant sequence under an assay primer and detection was impaired by approximately 10-fold. The strain 95 variant sequence, as well as another variant sequence with a more critical mismatch (detection impaired approximately 100-fold), have been identified in one or more clinical specimen(s) through post-market surveillance activities.
[c] HPeV serotype 5 was detected at a concentration of 5.0E+03 $TCID_{50}$/mL (10× LoD). No sequence-based evidence for impaired reactivity with serotype 5 was identified. The higher concentration required for detection of this isolate relative to others is likely a consequence of testing cultures quantified based on infectivity ($TCID_{50}$) rather than nucleic acid concentration.

Table 21. Yeast Isolates Tested and Detected by the BIOFIRE ME Panel[a]

Isolate/Source ID	Strain/Serotype Information	Isolate/Source ID	Strain/Serotype Information
C. neoformans		***C. gattii***	
ATCC 32045	Unknown serotype type strain, CBS 132	ATCC MYA-4560	Serotype B strain WM179, type VGI
ATCC 208821	Serotype A, strain H99 type strain of var. *grubii*	ATCC MYA-4094	Serotype B strain R272, type VGIIb
ATCC MYA-4564	Serotype A strain WM148, type VNI	ATCC MYA-4877	Unknown serotype strain R38, type VGIIc
ATCC MYA-4566	Serotype AD strain WM628, type VNIII	ATCC MYA-4562	Serotype B strain WM161, type VGIII
ATCC MYA-4567	Serotype D strain WM629, type VNIV	ATCC MYA-4563	Serotype C strain WM779, type VGIV

[a] All isolates were detected at a 3× LoD or lower concentration.

Analytical Specificity (Cross-Reactivity and Exclusivity)

The potential for non-specific amplification and detection by the BIOFIRE ME Panel assays (cross-reactivity) was evaluated by testing high concentrations of on-panel (identified by the ME Panel assays) and off-panel (not intended to be identified by the BIOFIRE ME Panel assays) organisms. Off-panel organisms were selected for testing based on a combination of several factors including (1) relatedness to the species detected by the panel (near-neighbors), (2) clinical relevance, (3) likelihood of being present in CSF and (4) genetic similarity to assay primers, as determined by *in silico* analyses.

In addition, *in silico* analysis was performed to identify potential cross-reacting sequences or organisms. The analysis indicated that the BIOFIRE ME Panel *Haemophilus influenzae* assay(s) may cross-react with other *Haemophilus* species (*H. aegyptius, H. haemolyticus, H. parainfluenzae, and H. sputorum*) and that the BIOFIRE ME Panel Enterovirus assay may detect many serotypes of closely related human Rhinoviruses. The *Haemophilus* species and rhinoviruses are primarily found in the upper respiratory tract and are rarely isolated from CSF. Lastly, sequence analysis predicted that the BIOFIRE ME Panel assay for detection of *Cryptococcus neoformans* and *C. gattii* would cross-react with a related *Cryptococcus* species (*C. amylolentus*) that does not infect humans.

Table 22 lists the off-panel bacteria, viruses, fungi, and protists that were tested with the BIOFIRE ME Panel. All organisms were tested at the highest concentration possible based on the concentration of the culture (typically ≥1.0×10^6 CFU/mL for bacteria, ≥1.0×10^4 units/mL for viruses, and ≥ 1.0×10^5 cells/mL for fungi and protists). The cross-reactivity with *Haemophilus* species, Rhinoviruses and *C. amylolentus* predicted by *in silico* analysis was confirmed in the testing (cross-reactivity indicated by bold font in Table 22). No other cross-reactivity was predicted or observed.

NOTE: Precautions should be taken when handling CSF specimens to avoid contamination with potentially cross-reactive commensal or pathogenic organisms of the respiratory tract, such as commensal Haemophilus species and Rhinovirus.

Table 22. Off-Panel Organisms Tested by the BIOFIRE ME Panel (cross-reactive organisms are in bold)

Gram-positive Bacteria	Gram-negative Bacteria	Viruses	
Bacillus cereus	*Acinetobacter baumannii*	Adenovirus A12	Rotavirus
Bacillus subtilis	*Acinetobacter lwoffii*	Adenovirus B35	Rubella Virus
Corynebacterium striatum	*Citrobacter freundii*	Adenovirus C2	St. Louis Encephalitis Virus
Corynebacterium urealyticus	*Citrobacter koseri*	Adenovirus D20	West Nile Virus
Enterococcus faecalis	*Cronobacter sakazakii*	Adenovirus E4	
Enterococcus faecium	*Elizabethkingia meningoseptica*	Adenovirus F41	
Listeria ivanovii	*Enterobacter cloacae*	BK polyoma virus	
Listeria innocua	*Escherichia coli* (non-K1)	Coronavirus 229E	
Mycobacterium tuberculosis	*Escherichia fergusonii*	Coronavirus NL63	**Fungi**
Cutibacterium (Propionibacterium) acnes	*Escherichia hermanii*	Coronavirus OC43	*Aspergillus fumigatus*
Staphylococcus aureus	*Escherichia vulneris*	Dengue virus (Type 2)	*Candida albicans*
Staphylococcus capitis	*Haemophilus ducreyi*	Epstein-Barr Virus	*Candida krusei*
Staphylococcus epidermidis	***Haemophilus aegyptius***[a,b]	Hepatitis B virus (HBV)	*Candida parapsilosis*
Staphylococcus haemolyticus	***Haemophilus haemolyticus***[a,b]	Hepatitis C virus (HCV)	*Candida tropicalis*
Staphylococcus hominis	*Haemophilus parahaemolyticus*	Human Herpesvirus 7	*Cryptococcus albidus*
Staphylococcus lugdunensis	***Haemophilus parainfluenzae***[a,c]	Human herpesvirus 8	***Cryptococcus amylolentus***[e]
Staphylococcus saprophyticus	***Haemophilus sputorum***[a,c]	Human Immunodeficiency Virus	*Cryptococcus laurentii*
Staphlyococcus warneri	*Klebsiella (Enterobacter) aerogenes*	**Human Rhinovirus A1**[d]	*Cryptococcus uniguttulatus*
Streptococcus anginosus	*Klebsiella pneumoniae*	**Human Rhinovirus A16**[d]	*Filobasidium capsuligenum*
Streptococcus bovis	*Morganella morganii*	**Human Rhinovirus B3**[d]	
Streptococcus dysgalactiae	*Neisseria meningitidis* (Unencapsulated)	**Human Rhinovirus B83**[d]	
Streptococcus intermedius	*Neisseria gonorrhoeae*	Influenza A H1N1	
Streptococcus mitis (*tigurinus*)	*Neisseria lactamica*	Influenza A H1N1-2009	**Pathogenic Protists**
Streptococcus mutans	*Neisseria mucosa*	Influenza A H3N2	*Naeglaria fowleri*
Streptococcus oralis	*Neisseria sicca*	Influenza B	*Toxoplasma gondii*
Streptococcus pseudopneumoniae	*Pantoea agglomerans*	Japanese Encephalitis Virus	
Streptococcus pyogenes	*Proteus mirabilis*	JC polyoma virus	
Streptococcus salivarius	*Pseudomonas aeruginosa*	La Crosse Encephalitis Virus	
Streptococcus sanguinis	*Salmonella bongori*	Measles Virus	
	Salmonella enterica	Mumps Virus	
	Serratia marcescens	Parainfluenza virus 1	
	Shigella boydii	Parainfluenza virus 2	
	Shigella flexneri	Parainfluenza virus 3	
	Shigella sonnei	Parainfluenza virus 4	
	Sphingomonas paucimobilis	Parvovirus B19	
	Treponema pallidum	Respiratory Syncytial Virus	

[a] Detected by the BIOFIRE ME Panel as *Haemophilus influenzae*.
[b] *H. aegyptius* and *H. haemolyticus* are closely-related species within the *H. influenzae* group and are generally described as commensal bacteria of the upper respiratory tract, rarely isolated from CSF. Cross-reactivity with *H. aegyptius* will be observed at all concentrations at or above LoD (>1.0E+03 CFU/mL) while cross-reactivity with *H. haemolyticus* was observed at concentrations >1.0E+05 CFU/mL.
[c] *H. parainfluenzae* and *H. sputorum* are closely-related species within the *H. parainfluenzae* group, and are generally described as commensal bacteria of the upper respiratory tract, rarely isolated from CSF. Cross-reactivity with these species was observed at concentrations >5.0E+06 CFU/mL.
[d] Detected by the BIOFIRE ME Panel as Enterovirus. Human Rhinoviruses are respiratory viruses and rarely isolated from CSF.
[e] Detected by the BIOFIRE ME Panel as *Cryptococcus neoformans/gattii*. *C. amylolentus* is not isolated from humans (normal habitat is insect frass).

Reproducibility

A multi-variable reproducibility study was performed on the BIOFIRE 2.0 system to determine the reproducibility of the BIOFIRE ME Panel results. The study incorporated a range of potential variation introduced by different operators testing on different systems/modules, days, and pouch lots (at least three). The samples tested contained a representative subset of nine different organisms (gram-positive and gram-negative bacteria, yeast, and DNA and RNA viruses) with each at three different concentrations (Negative, Low Positive (1× LoD), and Moderate Positive (3× LoD)). The multi-analyte samples were tested on five different days for 90 data points per sample.

A summary of results (percent (%) agreement with the expected result, or agreement between replicates) is provided in Table 23. The 'System' columns represent within-laboratory reproducibility and the 'All Systems' columns represent between-laboratory reproducibility.

NOTE: The reproducibility of the BIOFIRE ME Panel results on the BIOFIRE Torch system was evaluated in a similar study with equivalent results (≥95.6% agreement with the expected Detected and Not Detected results for Low Positive and Negative samples). Equivalent reproducibility of detection was also observed in a study performed on the original BIOFIRE FILMARRAY System, which is no longer in production.

Table 23. Reproducibility of the BIOFIRE ME Panel Test Results[a]

Test Result (Organism/Isolate Tested)	**Concentration Tested**	**Expected Result**	**System A**	**System B**	**System C**	**All Systems** (95% Confidence Interval)
BACTERIA						
***E. coli* K1** (ATCC 700973)	**Moderate Positive 3× LoD** 3.0E+03 CFU/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)
	Low Positive 1× LoD 1.0E+03 CFU/mL	**Detected**	**29/30** 96.7%	**29/30** 96.7%	**29/30** 96.7%	**87/90 96.70%** (90.6%-99.3%)
	Negative (No analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)
H. influenzae (ATCC 10211)	**Moderate Positive 3× LoD** 3.0E+03 CFU/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)
	Low Positive 1× LoD 1.0E+03 CFU/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)
	Negative (No analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)
L. monocytogenes (ATCC 13932)	**Moderate Positive 3× LoD** 3.0E+03 CFU/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)
	Low Positive 1× LoD 1.0E+03 CFU/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)
	Negative (No analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)
N. meningitidis	**Negative** (No analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**120/120** 100%	**360/360 100% (99.0%-100%)**
S. agalactiae (ATCC 13813)	**Moderate Positive 3× LoD**	**Detected**	**29/30** 96.7%	**30/30** 100%	**29/30** 96.7%	**88/90 97.80%**

Test Result (Organism/Isolate Tested)	Concentration Tested	Expected Result	System A	System B	System C	All Systems (95% Confidence Interval)
	3.0E+03 CFU/mL					(92.2%-99.7%)
	Low Positive 1× LoD 1.0E+03 CFU/mL	**Detected**	**29/30** 96.7%	**29/30** 96.7%	**30/30** 100%	**88/90 97.80%** (92.2%-99.7%)
	Negative (No analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)
S. pneumoniae	**Negative** (No analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**120/120** 100%	**360/360 100% (99.0%-100%)**
VIRUSES						
CMV	**Negative** (No analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**120/120** 100%	**360/360 100% (99.0%-100%)**
EV Coxsackievirus A9 (Zeptometrix 0810017CF)	**Moderate Positive 3× LoD**[b] 1.5E+01 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)
	Low Positive 1× LoD[b] 5.0E+00 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)
	Negative (No analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)
HSV-1	**Negative** (No analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**120/120** 100%	**360/360 100% (99.0%-100%)**
HSV-2 (Zeptometrix 0810006CF)	**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%	**30/30** 100%	**29/30** 96.7%	**89/90 98.90%** (94.0%-100%)
	Negative (No analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)
HHV-6	**Negative** (No analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**120/120** 100%	**360/360 100%** (99.0%-100%)
HPeV (Zeptometrix 0810147CF)	**Moderate Positive 3× LoD** 1.5E+03 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)
	Low Positive 1× LoD 5.0E+02 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)
	Negative (No analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)
VZV (Zeptometrix 0810171CF)	**Moderate Positive 3× LoD** 3.0E-01 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**30/30** 100%	**29/30** 96.7%	**89/90 98.90%** (94.0%-100%)
	Low Positive 1× LoD 1.0E-01 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)
	Negative (No analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)
YEAST						
C. neoformans/gattii	**Moderate Positive 3× LoD**	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%**

Test Result (Organism/Isolate Tested)	Concentration Tested	Expected Result	System A	System B	System C	All Systems (95% Confidence Interval)
C. gattii (ATCC MYA-4877)	3.00E+02 CFU/mL					(96.0%-100%)
	Low Positive 1x LoD 1.0E+02 CFU/mL	**Detected**	**30/30** 100%	**30/30** 100%	**28/30** 93.3%	**88/90 97.80%** (92.2%-99.7%)
	Negative (No analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)

[a] Reproducibility data were collected on BIOFIRE 2.0 systems. Equivalent reproducibility was observed for 1×LoD testing performed on the BIOFIRE Torch system (not shown).
[b] Multiple of LoD is based on the lowest concentration (in $TCID_{50}$/mL) detected for this isolate.

Interference

Potentially interfering substances that could be present in CSF samples or introduced during specimen collection and testing were evaluated for their effect on BIOFIRE ME Panel performance (e.g. detection of analytes near LoD). Each substance was added to contrived samples containing representative organisms (gram-positive and gram-negative bacteria, DNA/RNA viruses, and yeast) at concentrations approximately 3× LoD. The concentration of substance added to the samples was equal to or greater than the highest level expected to be in CSF specimens (based on reference concentrations for normal or meningitis/encephalitis CSF, as indicated in Table 24).

The majority of substances evaluated had no effect on the BIOFIRE ME Panel control assays or organism test results. Valid results were obtained and each organism was detected in samples containing relevant and/or elevated levels of endogenous substances such as lactate, glucose, proteins (≤ 15 mg/mL), white blood cells, human genomic DNA, and blood, in samples added to transport media, and in samples containing ethanol (see Table 24). Interference or damage to the sample was observed with high levels of protein (albumin ≥40 mg/mL; well above the clinically relevant protein levels of 5.0mg/mL) or with bleach at a concentration > 0.1% (v/v).

Table 24. Effect of Potentially Interfering Substances on the BIOFIRE ME Panel

Endogenous Substances	Reference Concentration for CSF		Concentration Tested	Interference Results
	Normal	Meningitis/Encephalitis		
Glucose	40-70 mg/dL[85] (0.4-0.7 mg/mL)	≤ 70 mg/dL (≤ 0.7 mg/mL)[86]	990 mg/dL (9.9 mg/mL)	No Interference
Lactate	10-20 mg/dL[85] (0.1-0.2 mg/mL)	> 30 mg/dL (> 0.3 mg/mL)[87]	220 mg/dL (2.2 mg/mL)	No Interference
Protein [Albumin][a,b]	45 mg/dL Total Protein (0.45 mg/mL)[86]	50-500 mg/dL Total Protein (0.5-5.0 mg/mL)[86]	1,500 mg/dL (15 mg/mL)	No Interference[b]
			500 mg/dL (5 mg/mL)	No Interference
			100 mg/dL (1 mg/mL)	No Interference
Immunoglobulin (IgG)	0-8.0 mg/dL (0.0-0.08 mg/mL)[88]	> 8.0 mg/dL (> 0.08 mg/mL)	1000 mg/dL (10 mg/mL)	No Interference
White Blood Cells (WBC)	0-20 cells/µL[89]	5-5,000 cells/µL[85]	10,000 cells/µL	No Interference
Human Genomic DNA[c]	≤ 0.068 ng/µL	≤ 17 ng/µL	20 ng/µL	No Interference
Human Whole Blood[c]	None[d]		10% (v/v)	No Interference
Hemoglobin	None[d]		200mg/dL (2 mg/mL)	No Interference
Transport Media	**Concentration Tested**			**Result**
Trans-Isolate (T-I) Medium	50% (v/v)			No Interference
Viral Transport Medium (VTM)	50% (v/v)			No Interference
Disinfectants	**Concentration Tested**			**Result**

Endogenous Substances	Reference Concentration for CSF		Concentration Tested	Interference Results
	Normal	Meningitis/Encephalitis		
Ethanol	7% (v/v)			No Interference
Bleach	1% (v/v) [570 ppm chlorine in sample]			Interference[e]
	0.1% (v/v) [57 ppm chlorine]			No Interference
	0.01% (v/v) [5.7 ppm chlorine]			No Interference

[a] Normal levels for albumin in CSF are between 0.0-0.27 mg/mL[87] or approximately half the total protein.
[b] Interference (Not Detected results for one or more analytes in a sample) was observed at albumin concentrations of 40-50mg/mL; which is substantially greater than the highest total protein levels expected in a CSF specimen.
[c] Additional detection of HHV-6 was observed. Detection of this viral DNA was suspected or confirmed to be a consequence of viral integration[90] rather than non-specific cross-reactivity or interference from the substance itself.
[d] Blood (and hemoglobin) may be present in CSF specimens due to a bloody tap (lumbar puncture) or subarachnoid hemorrhage.
[e] No organisms were detected despite normal function of the pouch control assays. Further investigation indicated the organisms were not detected due to bleach-associated damage (oxidation or other damage) to nucleic acids in the sample prior to testing.

NOTE: Due to the potential for interference from protein and the potential for specimen damage from bleach, care should be taken in the interpretation of negative BIOFIRE ME Panel test results from CSF specimens that may contain unusually high levels of proteins (>15 mg/mL) or specimens that may have been exposed to bleach prior to testing.

NOTE: Although transport media were not found to interfere with analyte detection by the BIOFIRE ME Panel, the panel has not been validated for use with CSF specimens in transport media.

Potentially competing or interfering viruses and other microorganisms were also evaluated for their effect on BIOFIRE ME Panel performance (Table 25). Contrived samples containing representative organisms at concentrations approximately 3× LoD were spiked with high concentrations of another virus, bacterium, or yeast to evaluate the potential for competitive inhibition or interference in multi-pathogen samples. Valid results were obtained and each 3× LoD organism was detected in samples containing a high level of an additional on-panel or off-panel organism.

Table 25. Effect of Potentially Competing or Interfering Viruses and Other Microorganisms on the BIOFIRE ME Panel

On-Panel Organism Tested	Concentration Tested	Results
Escherichia coli (K1)	1.02E+08 CFU/mL	No Inhibition/Interference
Coxsackievirus A9 (Enterovirus)	2.19E+05 $TCID_{50}$/mL	No Inhibition/Interference
Herpes simplex virus 1	1.95E+06 $TCID_{50}$/mL	No Inhibition/Interference
Cryptococcus neoformans	8.10E+05 CFU/mL	No Inhibition/Interference
Off-Panel Organism Tested	**Concentration Tested**	**Results**
Epstein-Barr virus	1.64E+09 $TCID_{50}$/mL	No Inhibition/Interference
Influenza A H1N1-2009	2.45E+04 $TCID_{50}$/mL	No Inhibition/Interference
Cutibacterium (Propionibacterium) acnes	1.12E+07 cells/mL	No Inhibition/Interference
Staphylococcus epidermidis	1.95E+07 CFU/mL	No Inhibition/Interference
Escherichia coli (non-K1)	1.38E+08 CFU/mL	No Inhibition/Interference
Staphylococcus aureus	8.55E+06 CFU/mL	No Inhibition/Interference
Candida albicans	1.01E+06 CFU/mL	No Inhibition/Interference

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		
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, cat. 1		Acute toxicity, cat. 4 & Skin irritation, cat. 2		Acute aquatic hazard, cat.1 & Long-term aquatic hazard, cat.1
European In Vitro Diagnostic Regulation (IVDR 2017/746)			**UK Medical Devices Regulation 2002**		
	European Union Conformity		UKCA – UK Conformity Assessed		
Manufacturer Symbols (BioFire Diagnostics, LLC)					
	European Union Product Importer		A BIOFIRE Meningitis/Encephalitis (ME) Panel product		
	BIOFIRE ME Panel				

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

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

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

UK CA 0086
bioMérieux UK Ltd
Chineham Gate
Crockford Lane
Basingstoke RG24 8NA
UK

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

References

1. Thigpen, M. C. *et al.* Bacterial meningitis in the United States, 1998-2007. *N. Engl. J. Med.* **364**, 2016–2025 (2011).
2. Versalovic, J. *Manual of clinical microbiology*. (ASM Press, 2011).
3. Kim, B. Y., Kang, J. & Kim, K. S. Invasion processes of pathogenic Escherichia coli. *Int. J. Med. Microbiol.* **295**, 463–470 (2005).
4. Gaschignard, J. *et al.* Neonatal Bacterial Meningitis: 444 Cases in 7 Years. *Pediatr. Infect. Dis. J.* **30**, 212–217 (2011).
5. Lu, C. H., Chang, W. N., Chuang, Y. C. & Chang, H. W. The prognostic factors of adult gram-negative bacillary meningitis. *J. Hosp. Infect.* **40**, 27–34 (1998).
6. Gower, D. J., Barrows, A. A., 3rd, Kelly, D. L., Jr & Pegram, S., Jr. Gram-negative bacillary meningitis in the adult: review of 39 cases. *South. Med. J.* **79**, 1499–1502 (1986).
7. Agrawal, A. & Murphy, T. F. Haemophilus influenzae infections in the H. influenzae type b conjugate vaccine era. *J. Clin. Microbiol.* **49**, 3728–3732 (2011).
8. Ladhani, S. *et al.* Invasive Haemophilus influenzae Disease, Europe, 1996-2006. *Emerging Infect. Dis.* **16**, 455–463 (2010).
9. Brouwer, M. C., van de Beek, D., Heckenberg, S. G. B., Spanjaard, L. & de Gans, J. Community-acquired Haemophilus influenzae meningitis in adults. *Clin. Microbiol. Infect.* **13**, 439–442 (2007).
10. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases.* (Elsevier).
11. Wing, E. J. & Gregory, S. H. Listeria monocytogenes: clinical and experimental update. *J. Infect. Dis.* **185 Suppl 1**, S18-24 (2002).
12. Disson, O. & Lecuit, M. Targeting of the central nervous system by Listeria monocytogenes. *Virulence* **3**, 213–221 (2012).
13. Vázquez-Boland, J. A. *et al.* Listeria pathogenesis and molecular virulence determinants. *Clin. Microbiol. Rev.* **14**, 584–640 (2001).
14. World Health Organization. Meningococcal meningitis fact sheet, February, 2015. *http://www.who.int/mediacentre/factsheets/fs141/en/*.
15. Johswich, K. O. *et al.* Invasive Potential of Nonencapsulated Disease Isolates of Neisseria meningitidis. *Infect Immun* **80**, 2346–2353 (2012).
16. Munro, R. Meningococcal disease: treatable but still terrifying. *Intern Med J* **32**, 165–169 (2002).
17. Pollard, A. J., Nadel, S., Ninis, N., Faust, S. N. & Levin, M. Emergency management of meningococcal disease: eight years on. *Arch. Dis. Child.* **92**, 283–286 (2007).
18. Cohn, A. C. *et al.* Prevention and control of meningococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* **62**, 1–28 (2013).
19. McIntyre, P. B., O'Brien, K. L., Greenwood, B. & van de Beek, D. Effect of vaccines on bacterial meningitis worldwide. *Lancet* **380**, 1703–1711 (2012).
20. Andersen, J., Christensen, R. & Hertel, J. Clinical features and epidemiology of septicaemia and meningitis in neonates due to Streptococcus agalactiae in Copenhagen County, Denmark: a 10 year survey from 1992 to 2001. *Acta Paediatr.* **93**, 1334–1339 (2004).
21. Verani, J. R., McGee, L., Schrag, S. J., & Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC). Prevention of perinatal group B streptococcal disease--revised guidelines from CDC, 2010. *MMWR Recomm Rep* **59**, 1–36 (2010).
22. Schrag, S. J. *et al.* Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. *N. Engl. J. Med.* **342**, 15–20 (2000).
23. Rodriguez-Granger, J. *et al.* Prevention of group B streptococcal neonatal disease revisited. The DEVANI European project. *Eur. J. Clin. Microbiol. Infect. Dis.* **31**, 2097–2104 (2012).
24. Heath, P. T. *et al.* Group B streptococcal disease in UK and Irish infants younger than 90 days. *Lancet* **363**, 292–294 (2004).
25. Dunne, D. W. & Quagliarello, V. Group B streptococcal meningitis in adults. *Medicine (Baltimore)* **72**, 1–10 (1993).
26. Domingo, P. *et al.* Group B streptococcal meningitis in adults: report of twelve cases and review. *Clin. Infect. Dis.* **25**, 1180–1187 (1997).
27. Brouwer, M. C., Tunkel, A. R. & van de Beek, D. Epidemiology, diagnosis, and antimicrobial treatment of acute bacterial meningitis. *Clin. Microbiol. Rev.* **23**, 467–492 (2010).
28. Scarborough, M. *et al.* Corticosteroids for bacterial meningitis in adults in sub-Saharan Africa. *N. Engl. J. Med.* **357**, 2441–2450 (2007).
29. Baraff, L. J., Lee, S. I. & Schriger, D. L. Outcomes of bacterial meningitis in children: a meta-analysis. *Pediatr. Infect. Dis. J.* **12**, 389–394 (1993).
30. Østergaard, C., Konradsen, H. B. & Samuelsson, S. Clinical presentation and prognostic factors of Streptococcus pneumoniae meningitis according to the focus of infection. *BMC Infect. Dis.* **5**, 93 (2005).
31. Centers for Disease Control and Prevention (CDC). Use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine among children aged 6-18 years with immunocompromising conditions: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb. Mortal. Wkly. Rep.* **62**, 521–524 (2013).
32. Musher, D. M. How effective is vaccination in preventing pneumococcal disease? *Infect. Dis. Clin. North Am.* **27**, 229–241 (2013).
33. Kothari, A., Ramachandran, V. G., Gupta, P., Singh, B. & Talwar, V. Seroprevalence of cytomegalovirus among voluntary blood donors in Delhi, India. *J Health Popul Nutr* **20**, 348–351 (2002).
34. Staras, S. A. S. *et al.* Seroprevalence of cytomegalovirus infection in the United States, 1988-1994. *Clin. Infect. Dis.* **43**, 1143–1151 (2006).
35. Rafailidis, P. I., Mourtzoukou, E. G., Varbobitis, I. C. & Falagas, M. E. Severe cytomegalovirus infection in apparently immunocompetent patients: a systematic review. *Virol. J.* **5**, 47 (2008).
36. Drew, W. L. Cytomegalovirus Disease in the Highly Active Antiretroviral Therapy Era. *Curr Infect Dis Rep* **5**, 257–265 (2003).
37. Centers for Disease Control and Prevention (CDC). Nonpolio enterovirus and human parechovirus surveillance --- United States, 2006-2008. *MMWR Morb. Mortal. Wkly. Rep.* **59**, 1577–1580 (2010).
38. Specter, S. *Clinical virology manual*. (ASM Press, 2009).
39. Cabrerizo, M. *et al.* Molecular characterization of enteroviruses associated with neurological infections in Spain, 2008. *J. Med. Virol.* **85**, 1975–1977 (2013).
40. Slika, S., Abbas, F. & Mahfouz, R. Implementation of the Cepheid Xpert EV assay for rapid detection of enteroviral meningitis: experience of a tertiary care center and a technical review. *Genet Test Mol Biomarkers* **17**, 232–235 (2013).
41. Dupuis, M. *et al.* Molecular detection of viral causes of encephalitis and meningitis in New York State. *J. Med. Virol.* **83**, 2172–2181 (2011).
42. Xu, F. *et al.* Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. *JAMA* **296**, 964–973 (2006).
43. Centers for Disease Control and Prevention (CDC). Seroprevalence of herpes simplex virus type 2 among persons aged 14-49 years--United States, 2005-2008. *MMWR Morb. Mortal. Wkly. Rep.* **59**, 456–459 (2010).
44. Wald, C. Persistence in the population: epidemiology, transmission. in *Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis* (eds. Arvin, A., Campadelli-Fiume, G. & Mocarski, E. et al) (Cambridge University Press, 2007).
45. Read, S. J. & Kurtz, J. B. Laboratory diagnosis of common viral infections of the central nervous system by using a single multiplex PCR screening assay. *J. Clin. Microbiol.* **37**, 1352–1355 (1999).
46. Salahuddin, S. Z. *et al.* Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science* **234**, 596–601 (1986).
47. Braun, D. K., Dominguez, G. & Pellett, P. E. Human herpesvirus 6. *Clin. Microbiol. Rev.* **10**, 521–567 (1997).

48. Arbuckle, J. H. *et al.* The latent human herpesvirus-6A genome specifically integrates in telomeres of human chromosomes in vivo and in vitro. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 5563–5568 (2010).
49. Aimola, G., Beythien, G., Aswad, A. & Kaufer, B. B. Current understanding of human herpesvirus 6 (HHV-6) chromosomal integration. *Antiviral Research* **176**, 104720 (2020).
50. Bates, M. *et al.* Predominant human herpesvirus 6 variant A infant infections in an HIV-1 endemic region of Sub-Saharan Africa. *Journal of Medical Virology* **81**, 779–789 (2009).
51. Álvarez-Lafuente, R., De las Heras, V., Bartolomé, M., Picazo, J. J. & Arroyo, R. Relapsing-Remitting Multiple Sclerosis and Human Herpesvirus 6 Active Infection. *Archives of Neurology* **61**, 1523 (2004).
52. Luppi, M., Barozzi, P., Maiorana, A., Marasca, R. & Torelli, G. Human herpesvirus 6 infection in normal human brain tissue. *J. Infect. Dis.* **169**, 943–944 (1994).
53. Hall, C. B. *et al.* Human herpesvirus-6 infection in children. A prospective study of complications and reactivation. *N. Engl. J. Med.* **331**, 432–438 (1994).
54. Chan, P. K., Ng, H. K., Hui, M. & Cheng, A. F. Prevalence and distribution of human herpesvirus 6 variants A and B in adult human brain. *J. Med. Virol.* **64**, 42–46 (2001).
55. Ogata, M. *et al.* Human Herpesvirus 6 (HHV-6) Reactivation and HHV-6 Encephalitis After Allogeneic Hematopoietic Cell Transplantation: A Multicenter, Prospective Study. *Clinical Infectious Diseases* **57**, 671–681 (2013).
56. Jumah, M. *et al.* COVID-19, HHV6 and MOG antibody: A perfect storm. *Journal of Neuroimmunology* **353**, 577521 (2021).
57. Stanway, G., Joki-Korpela, P. & Hyypiä, T. Human parechoviruses--biology and clinical significance. *Rev. Med. Virol.* **10**, 57–69 (2000).
58. Tauriainen, S. *et al.* Human parechovirus 1 infections in young children--no association with type 1 diabetes. *J. Med. Virol.* **79**, 457–462 (2007).
59. Westerhuis, B. *et al.* Human parechovirus seroprevalence in Finland and the Netherlands. *J. Clin. Virol.* **58**, 211–215 (2013).
60. Harvala, H., Wolthers, K. C. & Simmonds, P. Parechoviruses in children: understanding a new infection. *Curr. Opin. Infect. Dis.* **23**, 224–230 (2010).
61. Sharp, J. *et al.* Characteristics of young infants in whom human parechovirus, enterovirus or neither were detected in cerebrospinal fluid during sepsis evaluations. *Pediatr. Infect. Dis. J.* **32**, 213–216 (2013).
62. Walters, B. *et al.* Detection of human parechovirus (HPeV)-3 in spinal fluid specimens from pediatric patients in the Chicago area. *J. Clin. Virol.* **52**, 187–191 (2011).
63. Renaud, C. *et al.* Introduction of a novel parechovirus RT-PCR clinical test in a regional medical center. *J. Clin. Virol.* **51**, 50–53 (2011).
64. Verboon-Maciolek, M. A. *et al.* Human parechovirus causes encephalitis with white matter injury in neonates. *Ann. Neurol.* **64**, 266–273 (2008).
65. Pierik, J. G., Gumbs, P. D., Fortanier, S. A., Van Steenwijk, P. C. & Postma, M. J. Epidemiological characteristics and societal burden of varicella zoster virus in the Netherlands. *BMC Infectious Diseases* **12**, 110 (2012).
66. Bonanni, P. *et al.* Varicella vaccination in Europe – taking the practical approach. *BMC Medicine* **7**, 26 (2009).
67. Dworkin, R. H. *et al.* Recommendations for the management of herpes zoster. *Clin. Infect. Dis.* **44 Suppl 1**, S1-26 (2007).
68. Thomas, S. L. & Hall, A. J. What does epidemiology tell us about risk factors for herpes zoster? *Lancet Infect Dis* **4**, 26–33 (2004).
69. Yawn, B. P. & Gilden, D. The global epidemiology of herpes zoster. *Neurology* **81**, 928–930 (2013).
70. Mainka, C., Fuss, B., Geiger, H., Höfelmayr, H. & Wolff, M. H. Characterization of viremia at different stages of varicella-zoster virus infection. *J. Med. Virol.* **56**, 91–98 (1998).
71. Schünemann, S., Mainka, C. & Wolff, M. H. Subclinical reactivation of varicella-zoster virus in immunocompromised and immunocompetent individuals. *Intervirology* **41**, 98–102 (1998).
72. Gilden, D., Mahalingam, R., Nagel, M. A., Pugazhenthi, S. & Cohrs, R. J. Review: The neurobiology of varicella zoster virus infection. *Neuropathol. Appl. Neurobiol.* **37**, 441–463 (2011).
73. Hasimoto e Souza, L. K. *et al.* Clinical and microbiological features of cryptococcal meningitis. *Rev. Soc. Bras. Med. Trop.* **46**, 343–347 (2013).
74. Marr, K. A. Cryptococcus gattii as an important fungal pathogen of western North America. *Expert Rev Anti Infect Ther* **10**, 637–643 (2012).
75. McCulloh, R. J. *et al.* Cryptococcus gattii genotype VGI infection in New England. *Pediatr. Infect. Dis. J.* **30**, 1111–1114 (2011).
76. Centers for Disease Control and Prevention (CDC). Emergence of Cryptococcus gattii-- Pacific Northwest, 2004-2010. *MMWR Morb. Mortal. Wkly. Rep.* **59**, 865–868 (2010).
77. Hagen, F. *et al.* Autochthonous and Dormant *Cryptococcus gattii* Infections in Europe. *Emerging Infectious Diseases* **18**, 1618–1624 (2012).
78. Biosafety in Microbiological and Biomedical Laboratories. (2009).
79. Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline M29.
80. Adams, D. A. *et al.* Summary of Notifiable Diseases - United States, 2011. *MMWR Morb. Mortal. Wkly. Rep.* **60**, 1–117 (2013).
81. *CIFOR Analysis of State Legal Authorities*. http://www.cifor.us/.
82. Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; NCCLS Approved Guideline. (2006).
83. User Protocol for Evaluation of Qualitative Test Performance; NCCLS Approved Guideline. (2008).
84. Jaton, K., Ninet, B., Bille, J. & Greub, G. False-Negative PCR Result Due to Gene Polymorphism: the Example of Neisseria meningitidis. *Journal of Clinical Microbiology* **48**, 4590–4591 (2010).
85. Braunwald, E. *Principles of Internal Medicine*. (2001).
86. Solomon, T., Hart, I. J. & Beeching, N. J. Viral encephalitis: a clinician's guide. *Practical Neurology* **7**, 288–305 (2007).
87. R A Komorowski, S G Farmer, G A Hanson, and L L Hause. Cerebrospinal fluid lactic acid in diagnosis of meningitis. *J Clin Microbiol.* **8**, 89–92 (1978).
88. SFIN - Clinical: Cerebrospinal Fluid (CSF) IgG Index. http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/8009.
89. Seehusen, D. A., Reeves, M. M. & Fomin, D. A. Cerebrospinal fluid analysis. *Am Fam Physician* **68**, 1103–1108 (2003).
90. Morissette, G. & Flamand, L. Herpesviruses and Chromosomal Integration. *Journal of Virology* **84**, 12100–12109 (2010).

REVISION HISTORY

Version	Revision Date	Description of Revision(s)
01-04	N/A	Previous Revisions
05	June 2021	**Additions:** • Revision history table • EUDAMED link to the EU Summary of Safety and Performance required for compliance to the European In Vitro Diagnostic Regulation (IVDR 2017/746) • REACH regulation statement for EU customers • Adverse event reporting notice for EU customers in the EU • Intended Purpose, Intended User, and Use Environment statement • Limit of Detection – addition of data for WHO International Standards for CMV and HHV-6 • Analytical Reactivity and Analytical Specificity – additional isolate testing **Updates to:** • Symbols Glossary • E-labeling links • Fix typographical errors and formatting issues • Principle of the Procedure , Materials, Warnings, Precautions, External Controls, and Limitations sections in order to align with other BIOFIRE Panels and post-market input • Limitations section – re-ordered and revised some limitations for clarity, particularly regarding risks associated with false positive or false negative results • Branding and logo • EC REP Address • Limit of Detection section – updated notes and footnotes for clarity and notice of variant HSV-1 with altered LoD • Analytical Reactivity (Inclusivity) section – revisions to Table 18 - Table 20 for clarification of limitations, including finding from post-market surveillance and additional testing of specific types for *S. pneumoniae* and Enterovirus and H. influenzae genospecies *quentinii*. *H. influenzae* biogroup *aegyptius* re-classified as *H. aegyptius* and moved to Analytical Specificity Table 22. • Analytical Specificity (Cross-Reactivity and Exclusivity) section – revision to text and Table 22 to include cross-reactivity observed with *H. parainfluenzae* and new species *H. sputorum*. Added testing of: Japanese Encephalitis Virus*, E. faecalis*, *E. faecium*, *S. warneri*, *A. baumannii*, *A. lwoffii*, *E. meningoseptica*, *H. aegyptius*, *Sphingomonas paucimobilis*, Noted re-classification of *Propionibacterium acnes* as *Cutibacterium acnes* and *Enterobacter aerogenes* as *Klebsiella aerogenes.* • Inteference section – revised Table 24 to transfer non-relevant Albumin test concentrations from the table to footnotes. **Removals:** • BIOFIRE FilmArray (1st generation system) references and operation. Please refer to revision 04 for information on the operation of the BIOFIRE ME Panel with the BIOFIRE FilmArray. Reproducibility data collected on BIOFIRE FilmArray system from Table 23 and removal of Table 24 (Reproducibility Tm data table - BIOFIRE FilmArray and BIOFIRE 2.0 studies)
06	April 2022	**Additions:** • UKCA Symbol to cover and symbols glossary • UKCA Authorized Rep Address added • UDI Symbol to symbols glossary • ME symbol added to the symbols glossary • Additional Sample Buffer ampoule steps

Version	Revision Date	Description of Revision(s)
		Updates to: • Corrections of minor typographical errors • EC updated to EU in front of importer information • Minor edits for clarification **Removals:** • "DO NOT REFRIGERATE" statement from the "Reagent Storage, Handling and Stability" section.
07	August 2023	**Updates to:** • Corrections of minor typographical errors • Summary of Organisms section updated for *Neisseria meningitidis* to address unencapsulated strains of *N. meningitidis*. • Customer Technical Support Email • Summary of Organisms section and limitation #20 updated for clarification on Human herpesvirus 6. • Branding • Summary of Safety and Performance link

INDEX