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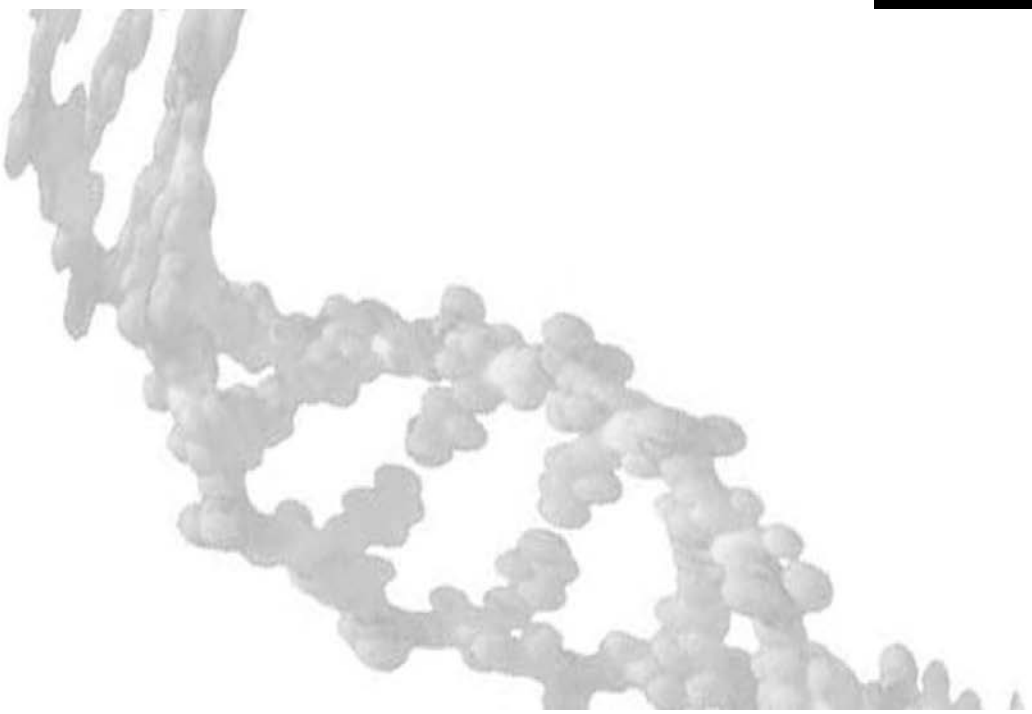

FilmArray® Meningitis/Encephalitis (ME) Panel

Instruction Booklet

IVD

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(801) 588-0507

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BioFire Diagnostics, LLC
390 Wakara Way
Salt Lake City, UT 84108
USA

EC REP

Qarad b.v.b.a
Cipalstraat 3
B-2440 Geel, Belgium

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TABLE OF SYMBOLS

The following symbols can be found on FilmArray ME Panel Kit components or throughout this Instruction Booklet. Use the definitions below as a guideline to interpreting the symbols.

Table of Symbols					
	Manufacturer		Catalog Number		Use By YYYY-MM-DD
	Consult Instructions for Use		Batch Code		Storage Temperature Limitations
	European Union Conformity		Serial Number		Contains Sufficient For <n> Tests
	*In vitro* Diagnostic Medical Device		Keep Away from Sunlight		Do Not Reuse
	Serious eye damage, cat. 1		Acute toxicity, cat. 4 & Skin irritation, cat. 2		Do Not Use if Package is Damaged

E-LABELING

The manual for this product can be accessed online at www.online-ifu.com/KEY-CODE. The product KEY-CODE is provided on the outer box label at the end of the URL. The KEY-CODE for this instruction booklet is also listed below. Additionally, a paper copy is available upon request by contacting customer service via phone, fax, e-mail, or regular mail.

FilmArray ME Panel CE IVD 6 and 30 Pack Kit - IFU	https://www.online-ifu.com/ITI0035
FilmArray ME Panel CE IVD 6 and 30 Pack Kit - Quick Guide	https://www.online-ifu.com/ITI0012
FilmArray ME Panel CE IVD 6 and 30 Pack Kit - SDS	https://www.online-ifu.com/ITI0067

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NAME AND INTENDED USE

FilmArray Meningitis/Encephalitis (ME) Panel

The FilmArray Meningitis/Encephalitis (ME) Panel is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with FilmArray and FilmArray 2.0 systems. The FilmArray 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 FilmArray 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 FilmArray 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 FilmArray 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 FilmArray 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 FilmArray ME Panel is not intended for testing of specimens collected from indwelling CNS medical devices.

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

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 FilmArray ME Panel conducts tests for the identification

of 14 potential CNS pathogens from CSF (Table 1). The specimen can be tested using the FilmArray ME Panel with results available within about one hour.

Table 1. Bacteria, Viruses, and Yeast Detected by the FilmArray 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, generally only encapsulated *N. meningitidis* are considered pathogenic. 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[15] 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%.[16] There are six licensed meningococcal vaccines available in US that may be used in persons of all ages, depending on the vaccine.[17] 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.[18]

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.[2,19] The most important risk factor for neonatal disease is maternal colonization with GBS.[2] Since 1996, CDC guidelines (updated in 2010)[20] have called for prophylactic antibiotic treatment several hours before delivery and have resulted in declining rates of neonatal GBS.[21] 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[22]. Mortality rates range from 10% for neonates[23] to 25-30% in adults.[24,25]

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.[26] Mortality approaches 50% in resource-poor countries, especially where HIV co-infection is a factor.[27] Neurological sequelae (cognitive impairment, deafness, epilepsy) are reported for up to 40% of survivors.[28,29] 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[30] and help reduce the risk of both invasive disease and pneumococcal pneumonia by 50-80%.[31]

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[32] and 36-90% in the US depending on age and race/ethnicity.[33] 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,[34] 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.[35] 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.[36] The most common EV serotypes are coxsackieviruses A9 and B1, and echoviruses 6, 9, and 18, which account for over 50% of serotyped detections. [36] Infections are spread via fecal-oral and respiratory routes and can spread quickly in community settings, particularly in

areas with poor sanitation.[37] 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.[38–40]

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.[37] In the U.S., overall seroprevalence for HSV-1 is around 60%.[41] The overall seroprevalence for HSV-2 is around 16% but varies with age, sex, and ethnicity.[42] 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.[43] 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,[44] 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.[40] 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,[45] when the rise of immunocompromised patients led to an increase in the population susceptible to severe disease outcome.[37] 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[46] and the infection establishes latency due to viral integration into host cells. 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.[47,48] This hypothesis is supported by the finding that HHV-6 inhabits CNS tissues, including the brain,[49] 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.[40] CNS disease associated with HHV-6 is found in both children and adults, suggesting CNS invasion during primary infection is possible.[46] While immunocompetent patients may experience CNS infection, it is much more common in severely immunosuppressed individuals.[37,46] 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.[50] 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[51] 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.[52] 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.

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.[53–55] As with EV, infections are spread via fecal-oral and respiratory routes with the most common symptoms being mild respiratory or gastrointestinal illness.[53] 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.[56] 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.[57–59] 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.[60]

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.[37] In the US, nearly 90% of the population had been infected with VZV before the advent of vaccines.[37] Similar rates have been reported in European countries.[61,62] Of those infected, between 10-30% develop zoster (a painful rash along the dorsal ganglia), primarily later in life.[63,64] It is estimated that the median global incidence of zoster is 4.0-4.5 per 1,000 person-years,[65] 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.[66,67] 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.[40] For immunocompromised patients, VZV neuronal illness can become chronic and lead to progressive deterioration and death.[63] 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.[68]

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.[2,69] *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.[70–73] 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.[69,72].

Principle of the Procedure

The FilmArray ME pouch is a closed system disposable that houses all the chemistry required to isolate, amplify and detect nucleic acid from multiple meningitis and encephalitis pathogens within a single CSF specimen obtained from a lumbar puncture. The rigid plastic component (fitment) of the FilmArray ME pouch contains reagents in freeze-dried form. The flexible plastic portion of the pouch is divided into discrete segments (blisters) where the required chemical processes are carried out. The user of the FilmArray ME Panel loads the sample into the FilmArray ME pouch, places the pouch into the FilmArray instrument, and starts the run. All other operations are automated.

The following is an overview of the testing procedure:

1. Remove the FilmArray pouch from its vacuum-sealed package. Since solutions are drawn into the FilmArray ME pouch by vacuum, it is important to keep pouches in their protective packaging until the time of use.
2. Place the FilmArray ME pouch into the FilmArray Pouch Loading Station. The FilmArray Pouch Loading Station has been designed to prevent error by providing instructions and visual cues in the form of color-coded arrows to ensure that the pouch is properly loaded.
3. Load Hydration Solution into the FilmArray ME pouch using the Hydration Injection Vial. The vial is fitted with a blunt stainless steel cannula, which is used to deliver the solution into the pouch. Loading the pouch with Hydration Solution rehydrates the freeze-dried reagents contained in the pouch fitment.
4. Squeeze Sample Buffer ampoule to deliver contents into Sample Injection Vial and add CSF specimen using Transfer Pipette. Tightly close the lid of the Sample Injection Vial and invert to mix. The Sample Buffer contains reagents that promote binding of nucleic acids to magnetic beads for isolation.

5. Load the sample/buffer mixture into the FilmArray ME pouch using the Sample Injection Vial. When the sample mixture is loaded, a process control contained in the fitment of the pouch is introduced into the sample. The process control monitors all of the critical processes that occur in the pouch.

6. Transfer the pouch to the instrument and initiate a run. The FilmArray software provides on-screen animations illustrating the steps needed to start the run.

7. View results on the test report at the completion of the run.

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

1. **Nucleic Acid Purification** - Nucleic acid purification occurs in the first three blisters of the pouch. The sample is lysed by agitation (bead beating) and the liberated nucleic acid is captured, washed and eluted using magnetic bead technology. These steps require about ten minutes and the bead-beater apparatus can be heard as a high-pitched whine during the first minute of operation.

2. **Reverse Transcription and 1st Stage Multiplex PCR** - Some pathogens identified by the FilmArray ME pouch are RNA viruses, and a reverse transcription (RT) step is performed to convert the viral RNA into cDNA prior to amplification. The purified nucleic acid solution is combined with a preheated master mix to initiate the RT step and subsequent thermocycling for multiplex PCR. The effect of 1st stage PCR is to enrich for the target nucleic acids present in the sample.

3. **2nd Stage PCR** - The products of 1st stage PCR are diluted and mixed with fresh PCR reagents containing an intercalating fluorescent DNA dye (LCGreen® Plus, BioFire Defense, LLC). This solution is distributed over the 2nd stage PCR array. The individual wells of the array contain primers for different assays (each present in triplicate) that target specific nucleic acid sequences from each of the pathogens detected, as well as control template material. These primers are 'nested' or internal to the specific products of the 1st stage multiplex reaction, which enhances both the sensitivity and specificity of the reactions.

4. **DNA Melting Analysis** – After 2nd stage PCR, the temperature is slowly increased and fluorescence in each well of the array is monitored and analyzed to generate a melt curve. The temperature at which a specific PCR product melts (melting temperature or T_m) is consistent and predictable and the FilmArray software automatically evaluates the data from replicate wells for each assay to report results. For a description of data interpretation and reporting see the Interpretation of Results section of this booklet.

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

MATERIALS PROVIDED

Each kit contains sufficient reagents to test 30 or 6 samples:

- Individually packaged FilmArray ME Panel pouches
- Single-use (1.0 mL) Sample Buffer ampoules
- Single-use pre-filled (1.5 mL) Hydration Injection Vials (blue)
- Single-use Sample Injection Vials (red)
- Individually packaged Transfer Pipettes

MATERIALS REQUIRED BUT NOT PROVIDED

FilmArray system including:

- FilmArray or FilmArray 2.0 instrument and software
- FilmArray Pouch Loading Station

WARNINGS AND PRECAUTIONS

General Precautions

1. For *in vitro* diagnostic use only.
2. This device is restricted to sale by or on the order of a physician, or to a clinical laboratory; its use is restricted to, by, or on the order of a physician.
3. A trained healthcare professional should carefully interpret the results from the FilmArray ME Panel in conjunction with a patient's signs and symptoms and results from other diagnostic tests.
4. FilmArray ME pouches are only for use with FilmArray systems.
5. Always check the expiration date on the pouch and do not use a pouch after its expiration date.
6. FilmArray pouches are stored under vacuum in individually-wrapped canisters. To preserve the integrity of the pouch vacuum for proper operation, be sure that a FilmArray Instrument 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,*[74] the CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections*,[75] or 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 is assigned the following classifications: Acute toxicity (Category 4), Serious Eye damage (Category 1), and Skin irritation (Category 2). Please refer to the FilmArray Reagent Kit Safety Data Sheet (SDS) for more information.
6. Sample Buffer will form hazardous compounds and fumes when mixed with bleach or other disinfectants.

WARNING: Bleach should never be added to Sample Buffer or sample waste.

Laboratory Precautions

1. **Preventing organism contamination**

Due to the sensitive nature of the FilmArray ME Panel, it is important to guard against contamination of the specimen and work area by carefully following the testing process outlined in this booklet, including these guidelines:

- Samples should be processed in a biosafety cabinet. If a biosafety cabinet is not used, a dead air box (e.g., AirClean PCR workstation), a splash shield (e.g., Bel-Art Scienceware Splash Shields), or a face shield should be used when preparing 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 FilmArray 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.
- Samples and pouches should be handled one at a time.
- Use clean gloves to remove materials from bulk packaging bags, and reseal bulk packaging bags when not in use.
- Change gloves and clean the work area between each sample.

2. **Preventing amplicon contamination**

A common concern with PCR-based assays is false positive results caused by contamination of the work area with PCR amplicon. Because the FilmArray 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.
- Avoid exposing pouches to sharp edges or anything that might cause a puncture.
- Use clean gloves to remove materials from bulk packaging bags, and reseal bulk packaging bags when not in use.

WARNING: If liquid is observed on the exterior of a pouch, the liquid and pouch should be immediately contained and discarded in a biohazard container. The instrument and work space must be decontaminated as described in the FilmArray Operator's Manual.

DO NOT PERFORM ADDITIONAL TESTING UNTIL THE AREA HAS BEEN DECONTAMINATED.

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.[76,77] 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.

REAGENT STORAGE, HANDLING AND STABILITY

1. Store the test kit, including reagent pouches and buffers, at room temperature (15–25 ºC). **DO NOT REFRIGERATE.**
2. Avoid storage of any materials near heating or cooling vents or in direct sunlight.
3. All kit components should be stored and used together. Do not use components from one kit with those of another kit. Discard any extra components from the kit after all pouches have been used.
4. Always check the expiration date and do not use reagents beyond the expiration date printed on the pouch or kit.
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 60 minutes).

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.

Minimum Sample Volume - 200 µL of CSF specimen is required for testing.

Transport and Storage - Specimens should be processed and tested with the FilmArray ME Panel as soon as possible, though they may be stored for up to one day at room temperature (approximately 23°C), or under refrigeration (approximately 4°C) for up to seven days.

PROCEDURE

Refer to the FilmArray Meningitis/Encephalitis Panel Quick Guide, the FilmArray Training Video, or the FilmArray 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 FilmArray ME pouch should be prepared at a time. Once sample is added to the pouch, it should be promptly transferred to the instrument to start the run. After the run is complete, the pouch should be discarded in a biohazard container.

Prepare Pouch

1. Thoroughly clean the work area and the FilmArray Pouch Loading Station with freshly prepared 10% bleach (or suitable disinfectant) followed by a water rinse.

2. Remove the pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective aluminum canister.

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

3. Slide the pouch into the FilmArray Pouch Loading Station so that the red and blue labels on the pouch align with the red and blue arrows on the FilmArray Pouch Loading Station.
4. Place a blue-capped Hydration Injection Vial in the blue well of the FilmArray Pouch Loading Station.
5. Place a red-capped Sample Injection Vial in the red well of the FilmArray Pouch Loading Station.

Hydrate Pouch

1. Twist and lift the Hydration Injection Vial, leaving blue cap in the well of the FilmArray Pouch Loading Station.
2. Insert the cannula tip into the port in the pouch located directly below the blue arrow of the FilmArray Pouch Loading Station. Push down forcefully in a firm and quick motion until you hear a faint “pop” and feel an ease in resistance. The correct volume of liquid will be pulled into the pouch by vacuum.
3. 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.

Prepare Sample Mix

1. Hold the Sample Buffer ampoule so that the tip is facing up.

   NOTE: Use care to avoid touching the tip during handling, as this may introduce contamination.

2. Gently pinch the textured plastic tab on the side of the ampoule until the seal snaps.
3. Invert the ampoule over the red-capped Sample Injection Vial and re-position thumb and forefinger to grip the bottom of the ampoule. Dispense Sample Buffer using a slow, forceful squeeze, followed by a second squeeze. Squeezing the ampoule additional times will generate excessive bubbles, which should be avoided.
4. Thoroughly mix the patient sample.
5. Using the Transfer Pipette provided in the test kit, draw cerebrospinal fluid (CSF) sample to the second line (approximately 0.2 mL) of the Transfer Pipette. Add the sample to the Sample Buffer in the Sample Injection Vial. Discard the Transfer Pipette in a biohazard waste container and tightly close the lid of the Sample Injection Vial.

   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 FilmArray Pouch Loading station and gently invert the vial at least 3 times to mix.
7. Return the Sample Injection Vial to the FilmArray Pouch Loading Station.

Load Sample Mix

1. Slowly twist the Sample Injection Vial so it loosens from its red cap and pause for 3-5 seconds. Lift the Sample Injection Vial, leaving the red cap in the well of the FilmArray Pouch Loading Station.
2. Insert the cannula tip into the port in the pouch fitment located directly below the red arrow of the FilmArray Pouch Loading Station. Push down forcefully in a firm and quick motion until you hear a faint “pop” and feel an ease in resistance. The correct volume of liquid will be pulled into the pouch by vacuum.
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 FilmArray Pouch Loading Station.

Run Pouch

1. Ensure that the FilmArray device is powered on and ready for use.
2. Follow on-screen instructions and procedures described in the Operator’s Manual to place the pouch in an instrument, enter pouch, sample and operator information, and start the run.

   Pouch identification (Lot Number and Serial Number), Pouch Type and Protocol are preprogrammed in the rectangular barcode located on the FilmArray pouch. The information will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, Pouch Type and Protocol can be manually entered from the information provided on the pouch label into the appropriate fields. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.
3. 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.
4. If necessary, select the appropriate protocol for your sample type from the Protocol drop down list.
5. Enter a user name and password in the Name and Password fields.

   NOTE: The bead-beater apparatus can be heard as a high-pitched noise (whine) during the first minute of operation.
6. Start run.
7. When the run is finished, follow the on-screen instructions to remove the pouch and immediately discard the pouch in a biohazard container.
8. The run file is automatically saved in the FilmArray database and the results 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 FilmArray 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 FilmArray 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 (80.2-84.2 for the RNA Process Control and 74.1-78.1 for the PCR2 Control). If required by local, state, or accrediting organization quality control requirements, users can monitor the system by trending Tm values for the control assays and maintaining records according to standard laboratory quality control practices.[78,79] Refer to FilmArray Operator's Manual for instructions on obtaining control assay Tm values. The PCR2 Control is used in all FilmArray pouch types (e.g., RP, GI, ME, and BCID) and can therefore be used to monitor the system when multiple pouch types are used on the same FilmArray system or instrument.

Good laboratory practice recommends running external positive and negative controls regularly. Molecular grade water, or artificial CSF, can be used as an external negative control. Previously characterized positive CSF samples or negative samples spiked with well characterized organisms can be used as external positive controls. External controls should be used in accordance with the appropriate accrediting organization requirements, as applicable.

INTERPRETATION OF RESULTS

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

Assay Interpretation

When 2nd stage PCR is complete, the FilmArray instrument performs a high resolution DNA melting analysis on the PCR products and measures the fluorescence signal generated in each well (for more information see FilmArray Operator's Manual). The FilmArray software then performs several analyses and assigns a final assay result. The steps in the analyses are described below.

Analysis of melt curves. The FilmArray software evaluates the DNA melt curve for each well of the 2nd stage PCR array to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then

the analysis software calculates the melting temperature (Tm) of the curve. The Tm value is then compared against the expected Tm range for the assay. If the software determines that the melt curve is positive and the Tm falls inside the assay-specific Tm range, the melt curve is called positive. If the software determines that the melt curve is negative or is not in the appropriate Tm range, the melt curve is called negative.

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 FilmArray 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 FilmArray ME Panel.

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

NOTE: The FilmArray 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.

FilmArray ME Panel Test Report

The FilmArray 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 FilmArray 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 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 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 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 FilmArray 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 Technical Support for further instruction.
Invalid	The controls are invalid because the run did not complete. (Typically this indicates a software or hardware error).	Note any error codes displayed during the run and the Run Status field in the Run Details section of the report. Refer to the FilmArray 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.	Accept the valid results of the repeat testing. If the error persists, contact 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 FilmArray Report,* for instruction.

LIMITATIONS

- For prescription use only.
- False negative results may occur when the concentration of organism(s) in the specimen is below the device limit of detection. In the prospective clinical study, two specimens were positive by standard of care culture and negative with the FilmArray ME Panel.
- 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.
- 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, Varicella, HHV-6 and *C. neoformans/gattii* were also established with retrospective clinical specimens.
- The FilmArray Meningitis/Encephalitis (ME) Panel performance has only been established on the FilmArray and FilmArray 2.0 systems.
- This test is a qualitative test and does not provide a quantitative value for the organism(s) in the specimen.
- Results from this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- The performance of this test has not been established for CSF specimens from patients without signs and/or symptoms of meningitis and/or encephalitis.
- The performance of this test has not been specifically evaluated for CSF specimens from immunocompromised individuals.
- The effect of antibiotic treatment on test performance has not been evaluated.
- The performance of this test has not been established for monitoring treatment of infection with any of the panel organisms.
- This test is not intended for use with CSF collected from indwelling medical devices (e.g., CSF shunts).
- CSF specimens should not be centrifuged before testing.
- The effect of interfering substances has only been evaluated for those listed in the labeling. Interference by substances other than those described in the Interference section below could lead to erroneous results.
- A negative FilmArray 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. There is a risk of false negative values due to the presence of sequence variants or rearrangements in the gene targets of the assay, procedural errors, inhibitors in specimens, technical error, sample mix-up, or infection caused by an organism not detected by the FilmArray ME Panel. Test results may also be affected by concurrent antimicrobial therapy or levels of organism in the sample that are below the limit of detection.
- The detection of organism nucleic acid is dependent upon proper sample collection, handling, transportation, storage, and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false positive and false negative results caused by improperly collected, transported, or

handled specimens. The RNA process control and the PCR 2 control will not indicate whether or not nucleic acid has been lost due to inadequate collection, transport or storage of specimens.

- Positive and negative predictive values are highly dependent on prevalence. False positive results are more likely for low prevalence analytes.

- Viral, bacterial, and yeast nucleic acid may persist *in vivo* independently of organism viability. Detection of organism targets does not imply that the corresponding organisms are infectious or the causative agents for clinical symptoms.

- HHV-6 or CMV can exist in latent form that is reactivated during infection due to other pathogens, including agents not detected by the FilmArray ME panel that may cause meningitis/encephalitis (e.g., *Mycobacterium tuberculosis* or HIV). When detected by the FilmArray ME, HHV-6 or CMV should be considered as the likely cause of meningitis/encephalitis only in appropriate clinical settings and following expert consultation.

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

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

- Some organisms detected by the FilmArray ME Panel, such as *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. Particular attention should be given to the Laboratory Precautions noted under the Warnings and Precautions section. Caution should also be exercised during specimen collection and testing to prevent contamination leading to false positive results.

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

- Cross-reactivity with organisms in addition to those listed in the Analytical Specificity section may lead to erroneous results. Cross-reactivity with human rhinoviruses may occur, but 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 associated with respiratory infections.

- Only *E. coli* strains possessing the K1 capsular antigen will be detected. All other *E. coli* strains/serotypes will not be detected.

- Only encapsulated strains of *N. meningitidis* will be detected. Unencapsulated *N. meningitidis* will not be detected.

EXPECTED VALUES

In the prospective clinical evaluation of the FilmArray 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 FilmArray ME Panel, stratified by age group, are presented in the following table. Overall, the FilmArray 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 FilmArray ME Panel) Summary by Age Group for the Prospective Clinical Evaluation (February through September 2014)

FilmArray 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. FilmArray 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 FilmArray 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 FilmArray 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 FilmArray 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 Performance

The clinical performance of the FilmArray 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 FilmArray 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 FilmArray ME Panel was evaluated by comparing the FilmArray 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 FilmArray 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 FilmArray 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 FilmArray ME Panel and reference/comparator method had a positive result for the specific analyte, and false negative (FN) indicates that the FilmArray 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 FilmArray ME Panel and the

reference/comparator method had negative results, and a false positive (FP) indicates that the FilmArray ME Panel result was positive but the comparator result was negative. The two-sided 95% confidence intervals were calculated.

Table 9. FilmArray ME 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 FilmArray 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 FilmArray 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 FilmArray 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	FilmArray 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 FilmArray ME Panel *C. neoformans/gattii* performance was PCR with bi-directional sequencing. FilmArray 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, FilmArray 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 FilmArray 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. FilmArray 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, FilmArray 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, FilmArray 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 FilmArray 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 a).

[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 a).

The FilmArray 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 FilmArray ME Panel

Co-detection Combination	Number of Specimens	Discrepant Analytes (detected by FilmArray 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 FilmArray 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 FilmArray 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 FilmArray 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 FilmArray ME 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. FilmArray 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 ME panel analytes by FilmArray 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 FilmArray testing are presented in Table 15.

Table 15. FilmArray 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	99.5	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

Analyte	/PPA			NPA		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
HHV-6	50/50	100	92.9-100	243/244	99.5	97.7-99.9
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.

FilmArray 2.0 Clinical Comparison

To demonstrate that performance of the FilmArray ME Panel when used with FilmArray 2.0 is equivalent to FilmArray, a combination of residual, de-identified CSF specimens and contrived CSF specimens covering all 14 analytes on the FilmArray 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 FilmArray testing are presented in Table 16 (FilmArray 2.0 results are represented in the numerator and FilmArray results are the denominator).

Table 16. Clinical Performance Comparison Summary of FilmArray ME Panel When Tested on FilmArray 2.0 (FA2.0) and 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%
	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 FilmArray ME Panel demonstrated 100% concordance for 18 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 FilmArray ME Panel analytes was estimated by testing dilutions of contrived samples containing known concentrations of one to five 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%) and LoD was confirmed for each analyte on FilmArray and FilmArray 2.0 systems.

The confirmed LoD for each FilmArray ME Panel analyte is listed in Table 17. For bacteria and yeast, LoD is provided in units of CFU/mL or cells/mL and for most viruses, LoD is provided in infectious units (tissue culture infectious dose or $TCID_{50}$/mL). For some viruses, LoD is (also) listed in nucleic acid copies/mL.

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

ME Panel Test Result	Species/Isolate Tested	LoD Concentration	Detection at LoD Concentration[a]
BACTERIA			
***E. coli* K1**	*E. coli* K1, strain C5 [Bort]; type O18ac:K1:H7 ATCC 700973	1×10^3 CFU/mL	20/20 100%
H. influenzae	*H. influenzae*, strain AMC 36-A-1 [572] type b, biotype I ATCC 10211	1×10^3 CFU/mL	20/20 100%
L. monocytogenes	*L. monocytogenes*, strain 1071/53, type 4b ATCC 13932	1×10^3 CFU/mL	20/20 100%
N. meningitidis	*N. meningitidis*, strain M-1574 [199/W135] ATCC 43744	100 CFU/mL	19/20 95%
S. agalactiae	*S. agalactiae*, type strain, G19, group B ATCC 13813	1×10^3 CFU/mL	20/20 100%
S. pneumoniae	*S. pneumoniae*, strain SV 1, serotype 1 ATCC 33400	100 cells/mL	19/20 95%
VIRUSES			
CMV	CMV, strain AD-169 Zeptometrix 0810003CF	100 $TCID_{50}$/mL (4.30×10^3 copies/mL)	20/20 100%
EV (Species A-D)	Coxsackievirus A6, species A, strain Gdula ATCC VR-1801	50 $TCID_{50}$/mL	20/20 100%
	Coxsackievirus A9, species B Zeptometrix 0810017CF	5 $TCID_{50}$/mL	20/20 100%
	Coxsackievirus A17, species C, strain G-12 ATCC VR-1023	5 $TCID_{50}$/mL	20/20 100%
	EV 70, species D, strain J670/71 ATCC VR-836	50 $TCID_{50}$/mL	20/20 100%
HSV-1	HSV-1, strain MacIntyre Zeptometrix 0810005CF	250 $TCID_{50}$/mL (1.51×10^3 copies/mL)	20/20 100%
HSV-2	HSV-2, strain MS Zeptometrix 0810006CF	50 $TCID_{50}$/mL (1.29×10^3 copies/mL)	20/20 100%
HHV-6	HHV-6A, strain U1102 NCPV 0003121v	1×10^4 copies/mL	19/20 95%
	HHV-6B, strain HST NCPV 0006111v	1×10^4 copies/mL	19/20 95%
HPeV	HPeV, type 3 Zeptometrix 0810147CF	500 $TCID_{50}$/mL	19/20 95%
VZV	VZV, strain Ellen Zeptometrix 0810171CF	0.10 $TCID_{50}$/mL (1.66×10^3 copies/mL)	20/20 100%
YEAST			
C. neoformans/gattii	*C. neoformans* var. *grubii*, type strain, H99 [H99JP, NYSD 1649] ATCC 208821	100 CFU/mL	20/20 100%
	C. gattii, strain A6MR38, AFLP6C, VGIIc ATCC MYA-4877	100 CFU/mL	20/20 100%

[a] Detection data are from LoD confirmation testing performed on the FilmArray system.

Analytical Reactivity (Inclusivity)

The analytical reactivity (inclusivity) of the FilmArray ME Panel was evaluated with a collection of 96 isolates that represent the diversity of the FilmArray ME Panel analytes. Isolates were selected to represent relevant species, subspecies, or serotypes. Isolates were initially tested at concentrations near (1-3×) the limit of detection (LoD), and all 98 isolates were detected by the FilmArray ME Panel at concentrations within 10× LoD. When possible, *in silico* analysis of

sequence data was used to make predictions of assay reactivity for less common strains or serotypes that were not tested but that may be detected by the FilmArray ME Panel.

A summary of ME Panel reactivity based on empirical data with notes about reactivity predictions based on *in silico* analysis is provided in Table 18 and all isolates tested are listed in Tables 19-20. No limitations on FilmArray ME Panel reactivity were identified.

Table 18. Summary of FilmArray ME Panel Analytical Reactivity (Inclusivity)

FilmArray ME Panel Test Result	# of Isolates Tested and Detected	Concentration Detected	Isolates Tested and Detected
Bacteria			
E. coli K1	5	1,000 - 3,000 CFU/mL	*E. coli* strains of the K1 serotype only
H. influenzae	9	1,000 - 3,000 CFU/mL	Non-typeable and typeable (types a-f) strains of *H. influenzae*
L. monocytogenes	6	1,000 - 3,000 CFU/mL	Types 1/2a, 1/2b, and 4b of *L. monocytogenes*[a]
N. meningitidis	7	100 - 300 CFU/mL	Encapsulated *N. meningitis* (serotypes W135, A, B, C, D, Y and DNA from a strains with a variant *ctrA* gene)
S. agalactiae	5	1,000 - 3,000 CFU/mL	Multiple serotypes or isolates of *S. agalactiae* (Group B *Streptococcus*)
S. pneumoniae	6	100 - 300 cells/mL	Multiple serotypes of *S. pneumoniae*
Viruses			
CMV	5	100 - 300 $TCID_{50}$/mL (4.3×10^3 - 1.3×10^4 copies/mL)	Multiple strains of Cytomegalovirus (CMV).
EV	18	5 - 50 $TCID_{50}$/mL	Representative isolates from all species (A-D) and several serotypes of human Enterovirus, Coxsackievirus, and Echovirus[b]
HSV-1	5	250 - 750 $TCID_{50}$/mL (1.5×10^3 - 4.5×10^3 copies/mL)	Multiple strains of Herpes simplex virus 1 (HSV-1)
HSV-2	5	50 - 150 $TCID_{50}$/mL (1.3×10^3 - 3.9×10^3 copies/mL)	Multiple strains of Herpes simplex virus 2 (HSV-2)
HHV-6	4	1×10^4 - 3×10^4 copies/mL	A and B variants of Human herpesvirus 6 (HHV-6)
HPeV	6	500 - 5,000 $TCID_{50}$/mL	Serotypes 1-6 of Human parechovirus (HPeV)[c]
VZV	5	0.1 - 0.3 $TCID_{50}$/mL (1.7×10^3- 5×10^3 copies/mL)	Multiple strains of Varicella zoster virus (VZV)
Yeast			
C. neoformans/gattii	10 (5 per species)	100 - 300 CFU/mL	Multiple strains, serotypes, and genotypes of *Cryptococcus neoformans* and *Cryptococcus gattii*

[a] *In silico* analysis of available sequences predicts that the FilmArray ME Panel will react with all currently characterized strains and serotypes of *L. monocytogenes*.

[b] *In silico* analysis of available sequences predicts that the FilmArray ME Panel will react with all currently characterized serotypes (>100) of human enteroviruses (including enteroviruses, coxsackieviruses, and echoviruses).

[c] Based on sequence analysis, the FilmArray ME Panel is also predicted to react with HPeV serotypes 7 and 8. No sequence data were available for predicting reactivity with other serotypes.

Table 19. Bacterial Isolates Tested Near (1-3×) LoD and Detected by the FilmArray ME Panel

Isolate	Strain/Serotype Information	Isolate 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 11116	Non-typeable, *aegyptius*	ATCC 13077	Serotype A
ATCC 9006	Type a [strain AMC 36-A-3]	ATCC 13090	Serotype B
ATCC 31512	Type b [strain Rab]	ATCC 13102	Serotype C
ATCC 10211	Type b [biotype 1]	ATCC 13113	Serotype D
ATCC 49699	Type c [strain C 9007]	ATCC 35561	Serotype Y
ATCC 9008	Type d [strain AMC 36-A-6]	*ctrA* variant	strain with variant *ctrA* gene[80]
ATCC 8142	Type e [strain AMC 36-A-7]		
ATCC 700223	Type f [strain GA-1264]		
L. monocytogenes		***S. pneumoniae***	
FSL-J2-020	Type 1/2a	ATCC 33400	Serotype 1
FSL-C1-056	Type 1/2a	ATCC BAA-334	Serotype 4
FSL-J2-064	Type 1/2b	ATCC BAA-341	Serotype 5
Clinical isolate – 2009 CDPH	Type 1/2b	NCTC 11900	Serotype 11A
FSL-J1-110	Type 4b	ATCC 700672	Serotype 14
ATCC 13932	Type 4b	ATCC 700673	Serotype 19A

Table 20. Viral Isolates Tested Near LoD (1-3×) and Detected by the FilmArray ME Panel

Isolate	Strain/Serotype Information	Isolate 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
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

Isolate	Strain/Serotype Information
NCPV 0812078v	Coxsackievirus B1
NCPV 0812142v	Coxsackievirus B2
Zeptometrix 0810074CF	Coxsackievirus B3
Zeptometrix 0810075CF	Coxsackievirus B4
Zeptometrix 0810019CF	Coxsackievirus B5
Zeptometrix 0810076CF	Echovirus 6
Zeptometrix 0810077CF	Echovirus 9
NCPV 0901047v	Echovirus 18
ATCC VR-1023	Coxsackievirus A17
ATCC VR-850	Coxsackievirus A21
ATCC VR-583	Coxsackievirus A24
ATCC VR-836	Enterovirus 70
Zeptometrix 0810237CF	Enterovirus 68 (aka Rhinovirus 87)

Isolate ID	Strain/Serotype Information
HHV-6	
NCPV 0003121v	6A - strain U1102
NCPV 0006111v	6B - strain HST
ATCC VR-1480	6B - strain SF
Zeptometrix 0810072CF	6B - strain Z29
HPeV	
Zeptometrix 0810145CF	Serotype 1
Zeptometrix 0810146CF	Serotype 2
Zeptometrix 0810147CF	Serotype 3
Zeptometrix 0810148CF	Serotype 4
Zeptometrix 0810149CF	Serotype 5[a]
Zeptometrix 0810150CF	Serotype 6
VZV	
Zeptometrix 0810171CF	Ellen (aka ATCC VR-1367)
Zeptometrix 0810172CF	Isolate A
Zeptometrix 0810173CF	Isolate B
Zeptometrix 0810168 CF	Strain 275
ATCC VR-916	Webster

[a] Detected at a concentration of 5,000 $TCID_{50}$/mL (10× LoD). All other serotypes tested were detected at 500-1,500 $TCID_{50}$/mL (1-3× LoD).

Table 21. Yeast Isolates Tested Near (1-3×) LoD and Detected by the FilmArray ME Panel

Isolate	Strain Information	Isolate ID	Strain 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

Analytical Specificity (Cross-Reactivity and Exclusivity)

The potential for non-specific amplification and detection by the FilmArray 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 ME Panel assays) organisms.

A total of 19 on-panel organisms (see Table 17 in the Limit of Detection section) and 107 off-panel organisms (see below) were tested, with additional *in silico* analysis to identify potential cross-reacting sequences or organisms. Off-panel organisms were selected for testing based on a combination of several factors including (1) relatedness to the species

detected by the ME Panel (near-neighbors), (2) clinical relevance, (3) likelihood of being present in CSF and (4) genetic similarity to ME Panel assay primers, as determined by *in silico* analyses.

Prior to testing, *in silico* analysis indicated that the FilmArray ME Panel *Haemophilus influenzae* assay(s) may cross-react with *Haemophilus haemolyticus* and that the FilmArray ME Panel Enterovirus assay may detect many serotypes of closely related human Rhinoviruses. Both *H. haemolyticus* and rhinoviruses are found in the upper respiratory tract but are rarely isolated from CSF.

Table 22 lists the off-panel bacteria, viruses, fungi, and protists that were tested with the FilmArray 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 *H. haemolyticus* and Rhinoviruses predicted by *in silico* analysis was confirmed by testing and cross-reactivity was also observed with *Cryptococcus amylolentus*, a near-neighbor to *Cryptococcus neoformans* that does not infect humans. 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.

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

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

Gram-positive Bacteria	Gram-negative Bacteria	Viruses	
Streptococcus sanguinis	*Serratia marcescens*	JC polyoma virus	
	Shigella boydii	La Crosse Encephalitis Virus	
	Shigella flexneri	Measles Virus	
	Shigella sonnei	Mumps Virus	
	Treponema pallidum		

[a] Detected by the FilmArray ME Panel as *Haemophilus influenzae*. *H. haemolyticus* is a commensal bacterium of the upper respiratory tract, rarely isolated from CSF. Cross-reactivity was observed only at concentrations > 1×10^5 CFU/mL.
[b] Detected by the FilmArray ME Panel as Enterovirus. Human Rhinoviruses are respiratory pathogens and rarely isolated from CSF.
[c] Detected by the FilmArray ME Panel as *Cryptococcus neoformans/gattii*. *C. amylolentus* is not isolated from humans (normal habitat is insect frass).

Reproducibility

Multi-center and multi-system reproducibility studies were performed on FilmArray and FilmArray 2.0 to determine the reproducibility of the FilmArray Meningitis/Encephalitis (ME) Panel. Reproducibility testing of contrived samples was performed at three test sites (FilmArray) or at one site on three systems (FilmArray 2.0). The study incorporated a range of potential variation introduced by site or system, day, operator (at least two per site or system), pouch lot (at least three), and instrument (at least three per site and six per system). The samples contained various combinations of nine different ME Panel analytes, each at three different concentrations (Negative, Low Positive, and Moderate Positive). Frozen samples were tested on five different days for 90 data points per sample on FilmArray and 90 data points per sample on FilmArray 2.0.

A summary of results (percent (%) agreement with the expected result) for each analyte (by site or system) is provided in Table 23. The reproducibility of Tm for each positive assay (within site or system) is provided in the Table 24.

The FilmArray ME Panel demonstrated similar reproducibility on FilmArray and FilmArray 2.0 for all analytes with a Tm standard deviation for each assay of ± 0.5°C or less.

Table 23. Reproducibility of the FilmArray ME Panel Test Results on FilmArray and FilmArray 2.0

Test Result (Organism/Isolate Tested)	**Concentration Tested**	**Expected Result**	**Agreement with Expected Result**							
			FilmArray				**FilmArray 2.0**			
			Site A	**Site B**	**Site C**	**All Sites** (95% Confidence Interval)	**System A**	**System B**	**System C**	**All Systems** (95% Confidence Interval)
BACTERIA										
***E. coli* K1** (ATCC 700973)	**Moderate Positive 3× LoD** 3×10^3 CFU/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)
	Low Positive 1× LoD 1×10^3 CFU/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)	**29/30** 96.7%	**29/30** 96.7%	**29/30** 96.7%	**87/90 96.7%** (90.6%-99.3%)
	Negative (No analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)
H. influenzae (ATCC 10211)	**Moderate Positive 3× LoD** 3×10^3 CFU/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)
	Low Positive 1× LoD 1×10^3 CFU/mL	**Detected**	**29/30** 96.7%	**30/30** 100%	**30/30** 100%	**89/90 98.9%** (94.0%-100%)	**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%)	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)
L. monocytogenes (ATCC 13932)	**Moderate Positive 3× LoD** 3×10^3 CFU/mL	**Detected**	**29/30** 96.7%	**30/30** 100%	**30/30** 100%	**89/90 98.9%** (94.0%-100%)	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)
	Low Positive 1× LoD 1×10^3 CFU/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)	**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%)	**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%)	**120/120** 100%	**120/120** 100%	**120/120** 100%	**360/360 100% (99.0%-100%)**
S. agalactiae (ATCC 13813)	**Moderate Positive 3× LoD** 3×10^3 CFU/mL	**Detected**	**29/30** 96.7%	**30/30** 100%	**27/30** 90.0%	**86/90 95.6%** (89.0%-98.8%)	**29/30** 96.7%	**30/30** 100%	**29/30** 96.7%	**88/90 97.8%** (92.2%-99.7%)

Test Result (Organism/Isolate Tested)	Concentration Tested	Expected Result	Agreement with Expected Result							
			FilmArray				FilmArray 2.0			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)	System A	System B	System C	All Systems (95% Confidence Interval)
	Low Positive 1× LoD 1×10^3 CFU/mL	**Detected**	**26/30** 86.7%	**30/30** 100%	**27/30** 90.0%	**83/90 92.2%** (84.6%-96.8%)	**29/30** 96.7%	**29/30** 96.7%	**30/30** 100%	**88/90 97.8%** (92.2%-99.7%)
	Negative (No analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)
S. pneumoniae	**Negative** (No analyte)	**Not Detected**	**118/120** 98.3%	**118/120** 98.3%	**118/120** 98.3%	**354/360**[a] **98.3%** (96.4%-99.4%)	**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%)**	**120/120** 100%	**120/120** 100%	**120/120** 100%	**360/360 100% (99.0%-100%)**
EV	**Moderate Positive 3× LoD** 15 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)
Coxsackievirus A9 (Zeptometrix 0810017CF)	**Low Positive 1× LoD** 5 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**30/30** 100%	**28/30** 93.3%	**88/90 97.8%** (92.2%-99.7%)	**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%)	**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%)**	**120/120** 100%	**120/120** 100%	**120/120**1 00%	**360/360 100% (99.0%-100%)**
HSV-2 (Zeptometrix 0810006CF)	**Moderate Positive 3× LoD** 150 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)
	Low Positive 1× LoD 50 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)	**30/30** 100%	**30/30** 100%	**29/30** 96.7%	**89/90 98.9%** (94.0%-100%)
	Negative (No analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)	**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%	**119/120** 99.2%	**120/120** 100%	**359/360 99.7%** (98.5%-100%)	**120/120** 100%	**120/120** 100%	**120/120** 100%	**360/360 100%** (99.0%-100%)

Test Result (Organism/Isolate Tested)	Concentration Tested	Expected Result	Agreement with Expected Result							
			FilmArray				FilmArray 2.0			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)	System A	System B	System C	All Systems (95% Confidence Interval)
HPeV (Zeptometrix 0810147CF)	**Moderate Positive 3× LoD** 1.5×10^3 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)
	Low Positive 1× LoD 500 $TCID_{50}$/mL	**Detected**	**29/30** 96.7%	**30/30** 100%	**30/30** 100%	**89/90 98.9%** (94.0%-100%)	**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%)	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)
VZV (Zeptometrix 0810171CF)	**Moderate Positive 3× LoD** 0.3 $TCID_{50}$/mL	**Detected**	**29/30** 96.7%	**30/30** 100%	**30/30** 100%	**89/90 98.9%** (94.0%-100%)	**30/30** 100%	**30/30** 100%	**29/30** 96.7%	**89/90 98.9%** (94.0%-100%)
	Low Positive 1× LoD 0.1 $TCID_{50}$/mL	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)	**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%)	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)
YEAST										
C. neoformans/gattii ***C. gattii*** (ATCC MYA-4877)	**Moderate Positive 30×[b] or 3× LoD** 3×10^3 CFU/mL (FilmArray) 300 CFU/mL (FilmArray 2.0)	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)
	Low Positive 10×[b] or 1x LoD 1×10^3 CFU/mL (FilmArray) 100 CFU/mL (FilmArray 2.0)	**Detected**	**30/30** 100%	**30/30** 100%	**30/30** 100%	**90/90 100%** (96.0%-100%)	**30/30** 100%	**30/30** 100%	**28/30** 93.3%	**88/90 97.8%** (92.2%-99.7%)
	Negative (No analyte)	**Not Detected**	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)	**60/60** 100%	**60/60** 100%	**60/60** 100%	**180/180 100%** (98.0%-100%)

[a] Six false positive *S. pneumoniae* results were reported. The unexpected results were observed at all three test sites, in different samples, on different days, and with different pouch lots. The overall incidence of false *S. pneumoniae* results observed in all reproducibility testing was <1%.

[b] *C. gattii* was tested at concentrations equivalent to 10× and 30× LoD for the combined *Cryptococcus neoformans/gattii* test result on the FilmArray system, while *C. gattii* was tested at the intended 1× and 3× LoD concentrations on the FilmArray 2.0 system.

Table 24. Reproducibility of Tm (°C) For Select FilmArray ME Panel Assays on the FilmArray and FilmArray 2.0

Test Result (Organism/Isolate Tested)	**Assay**	**Concentration Tested** (× LoD)	**Mean Tm (°C) (±StDev)**							
			FilmArray				**FilmArray 2.0**			
			Site A	**Site B**	**Site C**	**All Sites**	**System A**	**System B**	**System C**	**All Systems**
RNA Process Control	yeastRNA		82.4 **±0.2**	82.5 **±0.3**	82.0 **±0.2**	82.3 **±0.3**	81.7 **±0.3**	81.9 **±0.3**	81.7 **±0.3**	81.8 **±0.3**
PCR2 Control	PCR2		76.5 **±0.1**	76.6 **±0.3**	76.1 **±0.2**	76.4 **±0.3**	75.5 **±0.3**	75.7 **±0.3**	75.5 **±0.3**	75.6 **±0.3**
BACTERIA										
***E. coli* K1** (ATCC 700973)	Ecoli 3	**3× LoD**	82.9 **± 0.1**	82.8 **± 0.3**	82.6 **± 0.1**	82.8 **±0.3**	82.1 **±0.3**	82.3 **±0.3**	82.1 **±0.3**	82.2 **±0.3**
		1× LoD	83.0 **±0.1**	83.1 **±0.3**	82.6 **±0.2**	82.9 **± 0.3**	82.2 **±0.3**	82.4 **±0.3**	82.3 **±0.3**	82.3 **±0.3**
H. influenzae (ATCC 10211)	Hinfluenzae 1	**3× LoD**	78.6 **±0.1**	78.6 **±0.4**	78.2 **±0.2**	78.5 **±0.4**	77.9 **±0.3**	77.9 **±0.3**	77.7 **±0.2**	77.8 **±0.3**
		1× LoD	78.6 **±0.2**	78.7 **±0.3**	78.3 **±0.2**	78.5 **± 0.3**	78.0 **±0.3**	78.0 **±0.3**	77.9 **±0.3**	78.0 **±0.3**
	Hinfluenzae 2	**3× LoD**	82.0 **±0.1**	81.9 **±0.4**	81.6 **±0.2**	81.8 **± 0.3**	81.0 **±0.2**	81.1 **±0.2**	81.0 **±0.2**	81.0 **±0.2**
		1× LoD	82.0 **±0.1**	82.0 **±0.3**	81.7 **±0.2**	81.9 **± 0.3**	81.2 **±0.2**	81.3 **±0.2**	81.2 **±0.3**	81.2 **±0.2**
L. monocytogenes (ATCC 13932)	Lmonocytogenes	**3× LoD**	80.4 **±0.2**	80.6 **±0.3**	80.2 **±0.2**	80.4 **± 0.3**	80.0 **±0.4**	80.1 **±0.3**	80.0 **±0.3**	80.0 **±0.3**
		1× LoD	80.5 **±0.2**	80.6 **±0.4**	80.1 **±0.2**	80.4 **±0.4**	80.0 **±0.4**	80.1 **±0.3**	79.9 **±0.2**	80.0 **±0.3**
S. agalactiae (ATCC 13813)	Sagalactiae	**3× LoD**	82.0 **±0.1**	82.0 **±0.3**	81.6 **±0.2**	81.9 **± 0.3**	81.1 **±0.3**	81.3 **±0.3**	81.2 **±0.2**	81.2 **±0.3**
		1× LoD	82.1 **±0.1**	82.2 **±0.3**	81.7 **±0.2**	82.0 **± 0.3**	81.2 **±0.3**	81.4 **±0.2**	81.2 **±0.2**	81.3 **±0.3**
VIRUSES										
EV (Coxsackievirus A9) (Zeptometrix 0810017CF)	EV2	**3× LoD**	89.7 **±0.1**	89.7 **±0.3**	89.3 **±0.2**	89.6 **± 0.3**	89.1 **±0.3**	89.3 **±0.3**	89.2 **±0.3**	89.2 **±0.3**
		1× LoD	89.7 **±0.1**	89.7 **±0.4**	89.3 **±0.2**	89.6 **± 0.3**	89.1 **±0.3**	89.3 **±0.3**	89.2 **±0.2**	89.2 **±0.3**
HSV-2 (Zeptometrix	HSV2 1	**3× LoD**	75.6 **±0.3**	75.5 **±0.3**	75.1 **±0.3**	75.4 **± 0.3**	74.6 **±0.3**	74.8 **±0.3**	74.6 **±0.3**	74.7 **±0.3**

Test Result (Organism/Isolate Tested)	Assay	Concentration Tested (× LoD)	Mean Tm (°C) (±StDev)							
			FilmArray				FilmArray 2.0			
			Site A	Site B	Site C	All Sites	System A	System B	System C	All Systems
0810006CF)		1× LoD	75.9 ±0.2	76.0 ±0.2	75.4 ±0.3	75.7 ±0.4	74.9 ±0.3	75.1 ±0.3	74.9 ±0.3	75.0 ±0.3
	HSV2 2	3× LoD	88.8 ±0.2	88.9 ±0.4	88.4 ±0.2	88.7 ± 0.3	88.1 ±0.3	88.3 ±0.3	88.2 ±0.2	88.2 ±0.3
		1× LoD	88.9 ±0.2	89.0 ±0.3	88.5 ±0.2	88.8 ±0.4	88.2 ±0.3	88.5 ±0.3	88.3 ±0.2	88.3 ±0.3
HPeV (Zeptometrix 0810147CF)	HPeV	3× LoD	82.8 ±0.2	82.8 ±0.4	82.5 ±0.2	82.7 ± 0.3	82.2 ±0.3	82.3 ±0.3	82.1 ±0.2	82.2 ±0.3
		1× LoD	82.8 ±0.2	82.9 ±0.3	82.5 ±0.2	82.7 ± 0.3	82.3 ±0.3	82.3 ±0.2	82.2 ±0.3	82.3 ±0.3
VZV (Zeptometrix 0810171CF)	VZV 1	3× LoD	88.9 ±0.2	89.0 ±0.4	88.5 ±0.2	88.8 ±0.4	88.5 ±0.4	88.5 ±0.2	88.4 ±0.2	88.5 ±0.3
		1× LoD	89.0 ±0.2	89.0 ±0.5	88.5 ±0.2	88.8 ±0.4	88.5 ±0.3	88.5 ±0.3	88.3 ±0.2	88.4 ±0.3
	VZV 2	3× LoD	82.0 ±0.1	82.1 ±0.3	81.7 ±0.2	81.9 ± 0.3	81.5 ±0.3	81.5 ±0.2	81.4 ±0.2	81.5 ±0.2
		1× LoD	82.1 ±0.1	82.1 ±0.4	81.7 ±0.2	82.0 ± 0.3	81.5 ±0.3	81.5 ±0.2	81.4 ±0.2	81.5 ±0.2
YEAST										
C. neoformans/gattii (C. gattii) (ATCC MYA-4877)	Cryptococcus	30×[a] (FilmArray) 3× LoD (FilmArray 2.0)	82.0 ±0.1	82.0 ±0.3	81.6 ±0.2	81.8 ± 0.3	81.2 ±0.3	81.4 ±0.3	81.3 ±0.3	81.3 ±0.3
		10×[a] (FilmArray) 1× LoD (FilmArray 2.0)	82.0 ±0.2	82.1 ±0.2	81.6 ±0.2	81.9 ±0.3	81.3 ±0.3	81.5 ±0.4	81.3 ±0.2	81.4 ±0.3

[a] *C. gattii* was tested at concentrations equivalent to 10× and 30× LoD for the combined *Cryptococcus neoformans/gattii* test result on the FilmArray system, while *C. gattii* was tested at the intended 1× and 3× LoD concentrations on the FilmArray 2.0 system.

Interference

Potentially interfering substances that could be present in CSF samples or introduced during specimen collection and testing were evaluated for their effect on FilmArray ME Panel performance. Each substance was added to contrived samples containing representative ME Panel organisms at concentrations equivalent to 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 25).

The majority of substances evaluated had no effect on the FilmArray 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 25). Interference or damage to the sample was observed with high levels of protein (albumin >15 mg/mL) or with bleach at a concentration > 0.1% (v/v).

Table 25. Effect of Potentially Interfering Substances on the FilmArray ME Panel

Endogenous Substances	Reference Concentration for CSF		Concentration Tested	Interference Results
	Normal	Meningitis/Encephalitis		
Glucose	40-70 mg/dL[81] (0.4-0.7 mg/mL)	≤ 70 mg/dL (≤ 0.7 mg/mL)[82]	990 mg/dL (9.9 mg/mL)	No Interference
Lactate	10-20 mg/dL[81] (0.1-0.2 mg/mL)	> 30 mg/dL (> 0.3 mg/mL)[83]	220 mg/dL (2.2 mg/mL)	No Interference
Protein [Albumin][a]	45 mg/dL Total Protein (0.45 mg/mL)[82]	50-500 mg/dL Total Protein (0.5-5.0 mg/mL)[82]	5,000 mg/dL (50 mg/mL)	Interference [b]
			4,000 mg/dL (40 mg/mL)	Interference [b]
			1,500 mg/dL (15 mg/mL)	No Interference
			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)[84]	> 8.0 mg/dL (> 0.08 mg/mL)	1000 mg/dL (10 mg/mL)	No Interference
White Blood Cells (WBC)	0-20 cells/µL[85]	5-5,000 cells/µL[81]	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**
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[83] or approximately half the total protein.

[b] Interference (Not Detected results for one or more analytes in a sample) was observed only at concentrations substantially greater than the highest total protein levels expected in a CSF specimen.
[c] Additional detection of HHV-6 was observed. Detection of this virus was suspected or confirmed to be a consequence of viral integration[86] 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 FilmArray 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 FilmArray ME Panel testing, 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 FilmArray ME Panel performance (Table 26). Contrived samples containing representative ME Panel organisms at concentrations equivalent to approximately 3× LoD were spiked with high concentrations of other viruses, bacteria, or fungi that are detected by the ME Panel (competitive inhibition) or are not detected by the ME Panel (viral or microbial interference). Valid results were obtained and each 3× LoD ME Panel organism was detected in samples containing a high level of additional on-panel or off-panel organism.

Table 26. Effect of Potentially Competing or Interfering Viruses and Other Microorganisms on the FilmArray ME Panel

Competitive Inhibition	Concentration Tested	Results
Escherichia coli (K1)	1.02×10^8 CFU/mL	No Inhibition/Interference
Coxsackievirus A9 (Enterovirus)	2.19×10^5 $TCID_{50}$/mL	No Inhibition/Interference
Herpes simplex virus 1	1.95×10^6 $TCID_{50}$/mL	No Inhibition/Interference
Cryptococcus neoformans	8.10×10^5 CFU/mL	No Inhibition/Interference
Viral or Microbial Interference	**Concentration Tested**	**Results**
Epstein-Barr virus	1.64×10^9 $TCID_{50}$/mL	No Interference
Influenza A H1N1-2009	2.45×10^4 $TCID_{50}$/mL	No Interference
Proprionibacterium acnes	1.12×10^7 cells/mL	No Interference
Staphylococcus epidermidis	1.95×10^7 CFU/mL	No Interference
Escherichia coli (non-K1)	1.38×10^8 CFU/mL	No Interference
Staphylococcus aureus	8.55×10^6 CFU/mL	No Interference
Candida albicans	1.01×10^6 CFU/mL	No Interference

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FilmArray® ME Panel:
Estimated Limit of Detection for 1st WHO International Standard for Human Cytomegalovirus (HCMV)

TECHNICAL
::: NOTE

1. Introduction

The FilmArray Meningitis/Encephalitis (ME) Panel is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test 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.

Human Cytomegalovirus (HCMV) is one of several viruses associated with meningitis and/or encephalitis that can be detected in CSF by the FilmArray ME Panel.

The World Health Organization (WHO) oversees the development and distribution of International Biological Reference Preparations, which contain a defined biological activity or concentration of an analyte expressed in an internationally agreed unit. In October of 2010, the 1st WHO International Standard for Human Cytomegalovirus (HCMV) for Nucleic Acid Amplification Techniques was established [1] and it is currently available through the National Institute for Biological Standards and Control (NIBSC). The HCMV international standard (NIBSC code 09/162) is intended to be used in the standardization of nucleic acid amplification technique (NAT)-based assays for HCMV, allowing for direct comparisons between different methodologies and assays.

This technical note describes estimation of the FilmArray ME Panel Limit of Detection (LoD) for HCMV in international units (IU), as determined by testing of the 1st WHO International Standard for Human Cytomegalovirus. Currently, HCMV is the only analyte detected by the FilmArray ME Panel for which a WHO international standard for nucleic acid-based techniques is available.

2. Estimated LoD for Cytomegalovirus International Standard

FilmArray ME Panel testing was performed to determine an estimated LoD for human Cytomegalovirus (HCMV) in international units (IU). The reference material provided by NIBSC is a lyophilized whole virus preparation of the HCMV Merlin strain [2] (see also Analytical Reactivity

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(Table 20) in the FilmArray ME Panel Instruction Booklet [3]). When reconstituted according to the instructions for use provided with the material [4], the concentration of HCMV in the preparation is 5×10^6 IU/mL.

Once reconstituted, contrived samples were prepared by adding a known concentration of the HCMV standard to artificial CSF, followed by serial dilution to obtain five concentrations for testing (5×10^5 IU/mL, 5×10^4 IU/mL, 5×10^3 IU/mL, 5×10^2 IU/mL, and 5×10^1 IU/mL). A total of ten replicates at each concentration were tested using the FilmArray ME Panel on FilmArray and FilmArray 2.0 systems.

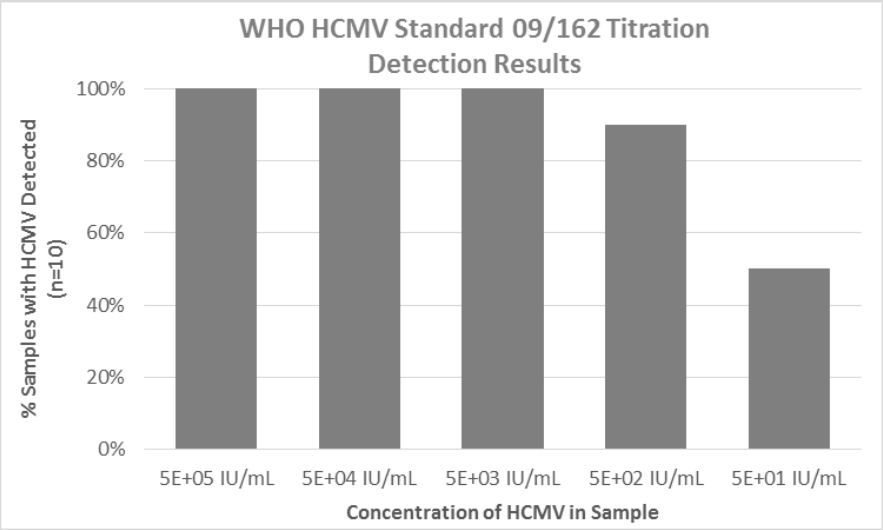

Figure 1. FilmArray ME Panel HCMV Detection in Contrived CSF Samples Containing Dilutions of the WHO HCMV International Standard 09/162

As shown in Figure 1, HCMV was detected in 100% of samples when tested at concentrations of 5×10^3 IU/mL and higher. HCMV was detected in 90% and 50% of samples when tested at concentrations of 5×10^2 IU/mL and 5×10^1 IU/mL, respectively. Therefore, the estimated FilmArray ME Panel LoD for HCMV (detection in ≥95% of samples) is 5×10^3 IU/mL.

For comparison, the confirmed LoD for a different HCMV isolate (strain AD-169) is 100 $TCID_{50}$/mL or 4.3×10^3 copies/mL, as determined by an alternate nucleic acid-based assay (see Table 1 below and the FilmArray ME Panel Instruction Booklet [3]).

Table 1. FilmArray ME Panel LoD for Human Cytomegalovirus

ME Panel Test Result	Species/Isolate Tested	LoD Concentration
CMV	CMV, strain AD-169 Zeptometrix 0810003CF	100 $TCID_{50}$/mL (4.30×10^3 copies/mL)
	CMV, strain Merlin NIBSC code 09/162	5×10^3 IU/mL[a]

[a] Represents the estimated LoD in IU/mL based on the lowest concentration of HCMV international standard detected in at least 95% of the ten replicates tested on FilmArray and FilmArray 2.0 systems.

3. Additional Information

Information about the reference material used in this testing is available from:

WHO International Laboratory for Biological Standards, UK Official Medicines Control Laboratory

National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, EN6 3QG T
+44 (0)1707 641000

http://www.nibsc.org/products/brm_product_catalogue/detail_page.aspx?catid=09/162

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Technical Support Contact Information

BioFire is dedicated to providing the best customer support available. If you have any questions or concerns about this process, please contact the FilmArray Technical Support team for assistance.

BioFire Technical Support
Email: support@biofiredx.com
Phone: +1-801-736-6354, select Option 5 and then Option 1

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