



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY ONLY**

**I Background Information:**

**A 510(k) Number**

K242256

**B Applicant**

QIAGEN GmbH

**C Proprietary and Established Names**

QIAstat-Dx Meningitis/Encephalitis (ME) Panel

**D Regulatory Information**

Product Code(s)	Classification	Regulation Section	Panel
PLO	Class II	21 CFR 866.3970 - Device To Detect And Identify Microbial Pathogen Nucleic Acids In Cerebrospinal Fluid	MI - Microbiology

**II Submission/Device Overview:**

**A Purpose for Submission:**

The purpose of this submission is to obtain substantial equivalence determination for the QIAstat-Dx ME Panel.

**B Measurand:**

Enterovirus, *Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis* (encapsulated), *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Cryptococcus neoformans/gattii* nucleic acid target sequences

**C Type of Test:**

A multiplexed nucleic acid real-time PCR test for use with the QIAstat-Dx Analyzer 1.0 instrument for the qualitative *in vitro* detection and identification of nucleic acid from bacteria, yeast, and viruses in a cerebrospinal fluid sample

### **III Intended Use/Indications for Use:**

#### **A Intended Use(s):**

See Indications for Use below.

#### **B Indication(s) for Use:**

The QIAstat-Dx Meningitis/Encephalitis (ME) Panel is a qualitative multiplexed nucleic acid real-time PCR based in vitro diagnostic test intended for use with the QIAstat-Dx Analyzer 1.0. The QIAstat-Dx ME Panel is capable of simultaneous detection and identification of multiple bacterial, viral, and yeast nucleic acids 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 QIAstat-Dx ME Panel: Enterovirus, Escherichia coli K1, Haemophilus influenzae, Listeria monocytogenes, Neisseria meningitidis (encapsulated), Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, and Cryptococcus neoformans/gattii\*.

The QIAstat-Dx ME Panel is indicated as an aid in the diagnosis of specific agents of meningitis and/or encephalitis and results must be used in conjunction with other clinical, epidemiological, and laboratory data. Results from the QIAstat-Dx 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 QIAstat-Dx ME Panel. The agent or agents detected may not be the definite cause of the disease. Negative results do not preclude central nervous system infection.

Not all agents of central nervous system infection are detected by this test and sensitivity in clinical use may differ from that described in the instructions for use.

The QIAstat-Dx ME Panel is not intended for testing specimens collected from indwelling central nervous system medical devices.

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

\*Cryptococcus neoformans and Cryptococcus gattii are not differentiated.

#### **C Special Conditions for Use Statement(s):**

Rx - For Prescription Use Only

#### **D Special Instrument Requirements:**

The QIAstat-Dx ME Panel assay is intended for use with the QIAstat-Dx Analyzer 1.0 instrument, which was originally cleared in K183597.

### **IV Device/System Characteristics:**

#### **A Device Description:**

QIAstat-Dx ME Panel

The QIAstat-Dx ME Panel is a single use plastic device which contains wet and dry reagents to allow automated extraction and reverse-transcription real-time quantitative polymerase chain reaction (RT-qPCR) for use in the QIAstat-Dx Analyzer 1.0 instrument. All reagents required to perform the test are pre-loaded on the cartridge, the microfluidics system is pneumatically operated preventing reagents within the cartridge from contacting the user or analyzer actuators.

Within the cartridge, multiple steps are automatically performed in sequence by using pneumatic pressure and a multiport valve to transfer sample and fluids via the transfer chamber to the intended destinations.

### QIAstat-Dx Analyzer 1.0 Instrument

The QIAstat-Dx Analyzer 1.0 instrument hosts the QIAstat-Dx ME Panel cartridge and runs predefined assay protocols by use of pneumatic pressure and a multiport valve.

### Interpretation of Results

The QIAstat-Dx Analyzer 1.0 instrument automatically interprets and saves test results. After ejecting the cartridge, the results summary screen is automatically displayed. Detected analytes (i.e., positive results) are displayed at the top of the list under the category 'Detected' in red font with a plus sign (+) next to the name. The last section of the results screen shows all targets tested with either a plus sign if it was detected or a minus sign (-) with the name in green colored font if the analyte was tested but not detected.

### Quality Control

The QIAstat-Dx ME Panel includes a titrated lyophilized MS2 bacteriophage internal control (IC) that provides verification that all analysis steps including sample resuspension, lysis, nucleic acid purification, reverse transcription, and PCR were successful. The results screen displays a message indicating that the internal controls "Passed" when the test was run successfully. A message of "Failed" indicates that the internal control was not amplified; 'Positive' test results are still reported as positive, but all 'Negative' results are invalid. Positive and negative external controls are recommended by the manufacturer but are not provided.

## **B Principle of Operation:**

Once the cartridge has been inserted into the instrument, the test starts automatically and runs for approximately 80 minutes. When the test is finished, the cartridge is removed by the user and discarded. The QIAstat-Dx Analyzer 1.0 instrument automatically interprets test results and displays a summary on the analyzer display screen. The results can be printed using a connected printer, if needed.

The collection of specimens and their subsequent loading into the QIAstat-Dx ME Panel cartridge should be performed by personnel trained in safe handling of biological samples. The user collects the cerebrospinal fluid (CSF) specimen and loads 200 µL of sample into the main port of the QIAstat-Dx ME Panel. All remaining steps through results interpretation and display are completed automatically by the QIAstat-Dx Analyzer 1.0 instrument.

**V Substantial Equivalence Information:**

**A Predicate Device Name(s):**

FilmArray Meningitis/Encephalitis (ME) Panel for use with FilmArray Torch

**B Predicate 510(k) Number(s):**

K160462

**C Comparison with Predicate(s):**

Device & Predicate Device(s):	<u>K242256</u>	<u>K160462</u>
Device Trade Name	QIAstat-Dx Meningitis/Encephalitis (ME) Panel	FilmArray Meningitis/Encephalitis (ME) Panel
General Device Characteristic Similarities		
Intended Use/Indications For Use	<p>The QIAstat-Dx Meningitis/Encephalitis (ME) Panel is a qualitative multiplexed nucleic acid real-time PCR based in vitro diagnostic test intended for use with the QIAstat-Dx Analyzer 1.0 instrument. The QIAstat-Dx ME Panel is capable of simultaneous detection and identification of multiple bacterial, viral, and yeast nucleic acids from cerebrospinal fluid (CSF) specimens obtained via lumbar puncture from individuals with signs and/or symptoms of meningitis and/or encephalitis.</p> <p>The following organisms are identified using the QIAstat-Dx ME Panel: Enterovirus, <i>Escherichia coli</i> K1, <i>Haemophilus</i></p>	<p>The FilmArray Meningitis/Encephalitis (ME) Panel is a qualitative multiplexed nucleic acid-based in vitro diagnostic test intended for use with FilmArray, FilmArray 2.0, and FilmArray Torch 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.</p> <p>The FilmArray ME Panel is indicated as an aid in the diagnosis of specific agents of meningitis and/or</p>

	<p><i>influenzae</i>, <i>Listeria monocytogenes</i>, <i>Neisseria meningitidis</i> (encapsulated), <i>Streptococcus agalactiae</i>, <i>Streptococcus pneumoniae</i>, <i>Streptococcus pyogenes</i>, and <i>Cryptococcus neoformans/gattii</i>*.</p> <p>The QIAstat-Dx ME Panel is indicated as an aid in the diagnosis of specific agents of meningitis and/or encephalitis and results must be used in conjunction with other clinical, epidemiological, and laboratory data. Results from the QIAstat-Dx 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 QIAstat-Dx ME Panel. The agent or agents detected may not be the definite cause of the disease. Negative results do not preclude central nervous system infection.</p> <p>Not all agents of central nervous system infection are detected by this test and</p>	<p>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.</p> <p>The FilmArray ME Panel is not intended for testing of specimens collected from indwelling CNS medical devices. The FilmArray ME Panel is intended to be used in conjunction with standard of care culture for organism recovery, serotyping, and antimicrobial susceptibility testing.</p>
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	<p>sensitivity in clinical use may differ from that described in the instructions for use.</p> <p>The QIAstat-Dx ME Panel is not intended for testing specimens collected from indwelling central nervous system medical devices.</p> <p>The QIAstat-Dx ME Panel is intended to be used in conjunction with standard of care culture for organism recovery, serotyping, and antimicrobial susceptibility testing.</p> <p><i>*Cryptococcus neoformans</i> and <i>Cryptococcus gattii</i> are not differentiated.</p>	
<b>Targets</b>	<p>Bacteria:</p> <ul style="list-style-type: none"> <li>• <i>Escherichia coli</i> K1</li> <li>• <i>Haemophilus influenzae</i></li> <li>• <i>Listeria monocytogenes</i></li> <li>• <i>Neisseria meningitidis</i> (encapsulated)</li> <li>• <i>Streptococcus agalactiae</i></li> <li>• <i>Streptococcus pneumoniae</i></li> </ul> <p>Virus:</p> <ul style="list-style-type: none"> <li>• Enterovirus</li> </ul> <p>Yeast:</p> <ul style="list-style-type: none"> <li>• <i>Cryptococcus neoformans/gattii</i></li> </ul>	<p>Bacteria:</p> <ul style="list-style-type: none"> <li>• <i>Escherichia coli</i> K1</li> <li>• <i>Haemophilus influenzae</i></li> <li>• <i>Listeria monocytogenes</i></li> <li>• <i>Neisseria meningitidis</i> (encapsulated)</li> <li>• <i>Streptococcus agalactiae</i></li> <li>• <i>Streptococcus pneumoniae</i></li> </ul> <p>Viruses:</p> <ul style="list-style-type: none"> <li>• Enterovirus</li> </ul> <p>Yeast:</p> <ul style="list-style-type: none"> <li>• <i>Cryptococcus neoformans/gattii</i></li> </ul>
<b>Specimen Type</b>	Cerebrospinal Fluid	Cerebrospinal Fluid
<b>Analyte Detected</b>	RNA/DNA	RNA/DNA
<b>Technology</b>	RT-PCR	RT-PCR

General Device Characteristic Differences		
Assay Controls	One internal processing control in each cartridge is subjected to all nucleic acid extraction and amplification steps.	Two controls are provided in each reagent pouch to control for sample processing and both stages of PCR and melt analysis. Labeling recommends use of negative (transport medium) and positive (previously characterized positive sample or negative sample spiked with target organism) external controls.
Targets	Bacterium: • <i>Streptococcus pyogenes</i>	Viruses: • Cytomegalovirus • Herpes simplex virus 1 • Herpes simplex virus 2 • Human herpesvirus 6 • Human parechovirus • Varicella zoster virus
Instrument	QIAstat-Dx Analyzer 1.0	FilmArray, FilmArray 2.0, and FilmArray Torch systems

## VI Standards/Guidance Documents Referenced:

- CLSI EP07 3<sup>rd</sup> Edition 7-275 Interference Testing in Clinical Chemistry
- CLSI EP17-A2 7-233 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition
- CLSI EP25-A (Replaces EP25-P) 7-235 Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline
- CLSI EP12-A2 7-152 User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline – Second Edition
- CLSI EP05-A3 (Reaffirmed: September 2019) 7-251 Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition
- CLSI MM03-3<sup>rd</sup> Edition (Replaces MM03-A2) 7-260 Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline
- IEC 62304 Edition 1.1 2015-06 CONSOLIDATED VERSION 13-79 Medical device software – Software life cycle processes

## VII Performance Characteristics (if/when applicable):

### A Analytical Performance:

#### 1. Inclusivity

- a. The Inclusivity (analytical reactivity) study extended the list of pathogen strains tested during the QIAstat-Dx ME Panel Limit of Detection (LoD) study to confirm the reactivity of the detection system in the presence of different strains of the same organisms at a concentration near or above the respective LoD.

A variety of clinically relevant strains of each target organism of the QIAstat-Dx ME Panel representing organism sub-types, strains, serotypes, and genotypes of different temporal and geographic diversity of each analyte were included in the study. Analytical reactivity was assessed with both *in vitro* wet-testing and *in silico* analysis.

A total of 130 strains covering 10 different analytical strains for each targeted organism or relevant species were tested as described in Table 1 below.

**Table 1: Inclusivity Strains Evaluated Wet Testing**

Target	Source (Catalog ID)	Strain/Serotype	Lowest Concentration Tested
<i>Escherichia coli</i> K1	ATCC (700973) <sup>a</sup>	Strain C5 [Bort]; O18ac:K1:H7	1x LoD
	ATCC (11775) <sup>a</sup>	NCTC 9001. Serovar O1:K1:H7	1x LoD
	NCTC (9007)	Strain Bi 7509/41; O7:K1:H-	0.03x LoD
	ATCC (23509)	NCDC Bi 7509-41 Serotype O7	1:100 from stock
	ATCC (23511)	:K1(L) :NM	
	ATCC (23511)	NCDC F 11119-41	0.03x LoD
	BEI Resources (NR- 17666)	O-2, U9-41	1:100 from stock
	BEI Resources (NR- 17674)	O-16, F1119-41	1:100 from stock
	ZeptoMetrix (0801905)	Z136 CTX-M-15	3x LoD
	NCTC (11101)	Sc15 02:K1:H6	3x LoD
	NCTC (9045)	Strain H61; O45:K1:H10	3x LoD
<i>Listeria monocytogenes</i>	ZeptoMetrix (0801534) <sup>a</sup>	Type 1/2b	1x LoD
	ATCC (19115) <sup>a</sup>	Type 4b. Strain Li 2	1x LoD
	ATCC (BAA-2659)	Type 1/2a. Strain 2011L-2676	3x LoD
	ATCC (19111)	Type 1/2a. Strain Li 20	3x LoD
	ZeptoMetrix (0804339) <sup>c</sup>	Type 4b	0.03x LoD <sup>c</sup>
	ATCC (13932)	serotype 4b. Strain 1071/53 [LMG 21264, NCTC 10527]	0.011x LoD
	ATCC (19114)	Li 23. Serotype 4a	0.02x LoD
	BEI Resources (NR- 13237)	FSL J2-064	1:100 from stock
	ATCC (7644)	Gibson	2x LoD
	ATCC (BAA-679)	EGDe	0.026x LoD
	ATCC (13090) <sup>a</sup>	Serotype B. M2092 [CIP 104218,	1x LoD
		L. Cunningham]	

<i>Neisseria meningitidis</i> (encapsulated)	ATCC (35561) <sup>a</sup>	Serotype Y. M-112 [BO-6]	1x LoD
	ATCC (13077)	Serogroup A, M1027 [NCTC10025]	0.03x LoD
	ATCC (13102)	Serogroup C, M1628	0.03x LoD
	ATCC (13113)	Serotype D. M158 [37A]	3x LoD
	IDT (gBlock 77859371) <sup>b</sup>	sequence with variant <i>ctrA</i> gene	1:1000 from stock
	ATCC (43744)	W135	0.03x LoD
	ATCC (BAA-335)	MC58	0.03x LoD
	ATCC (23255)	79 Eur. Serogroup B	1:1000 from stock
	ATCC (13092)	Serotype B. M997 [S-3250-L]	1:1000 from stock
<i>Streptococcus agalactiae</i>	ZeptoMetrix (0801545) <sup>a</sup>	Z019	1x LoD
	ATCC (13813) <sup>a</sup>	G19 group B	1x LoD
	ATCC (12403)	Serotype III. Typing strain D136C(3) [3 Cole 106, CIP 82.45]	1.2x LoD
	ATCC (BAA-611)	2603 V/R. Serotype V	0.013x LoD
	ATCC (31475)	type III-ST283	3x LoD
	BEI Resources (NR- 43898)	MNZ929	1:100 from stock
	ATCC (12401)	Typing strain H36B – type Ib	1.4x LoD
	ATCC (27591)	CDC SS700 [A909; 5541], type 1c	0.014x LoD
	ATCC (49446)	3139 [CNCTC 1/82] Serotype IV	0.0127x LoD
	ZeptoMetrix (0801556)	Z023	3x LoD
<i>Streptococcus pneumoniae</i>	ZeptoMetrix (0801439) <sup>a</sup>	19F	1x LoD
	ATCC (33400) <sup>a</sup>	Serotype 1. NCTC 7465	1x LoD
	ATCC (BAA-334)	Serotype 4. TIGR4 [JNR.7/87]	0.03x LoD
	ATCC (BAA-341)	Serotype 5. SPN1439-106	0.03x LoD
		[Colombia 5-19]	
	ATCC (10343)	Serotype 11A. Type 43	3x LoD
	ATCC (700672)	Serotype 14. VH14	0.03x LoD
	ATCC (700673)	Serotype 19A. Hungary 19A-6 [HUN663]	0.026x LoD
	ZeptoMetrix (0804016)	Z319; 12F	3x LoD
	ATCC (6303)	Diplococcus pneumoniae; Type 3.	3x LoD
		Strain [CIP 104225]	
	ATCC (BAA-661)	DCC1476 [Sweden 15A-25]	0.03x LoD
<i>Streptococcus pyogenes</i>	ZeptoMetrix (0804351) <sup>a</sup>	Z472; Serotype M1	1x LoD
	ATCC (19615) <sup>a</sup>	Bruno [CIP 104226]	1x LoD
	ZeptoMetrix (0801512)	Z018; Serotype M58	3x LoD
	ATCC (BAA-947)	Serotype M1. MGAS 5005	0.03x LoD
	ATCC (14289)	Lancefield's group A / C203 S	3x LoD
	ATCC (12203)	NCTC 8709 (Type 6 glossy)	0.03x LoD
	ATCC (12353)	Group a, type 12. Typing strain T12 [F. Griffith SF 42]	1:1000 from stock
	ATCC (12972)	Group a, type 14	0.03x LoD
	ATCC (8133)	Group a, type 23	3x LoD
	ATCC (12384)	C203 -Type 3	0.029x LoD
Enterovirus A	ZeptoMetrix (0810107CF) <sup>a</sup>	Coxsackievirus A16	1x LoD
	ATCC (VR-1801) <sup>a</sup>	A6, species A. Strain Gdula	1x LoD

	ATCC (VR-168)	A10. M.K. (Kowalik)	3x LoD
	ATCC (VR-1432)	Enterovirus 71. Strain H	3x LoD
	ZeptoMetrix (0810236CF)	Species A, Serotype EV-A71 (2003 Isolate)	1:100 from stock
	BEI Resources (NR-471)	Tainan/4643/1998	3x LoD
	ATCC (VR-1550) <sup>c</sup>	A2 Fl [Fleetwood]	3x LoD <sup>c</sup>
	ATCC (VR-673)	A7 – 275/58	3x LoD
	ATCC (VR-170)	A12 – Texas 12	1:100 from stock
	ATCC (VR-1775)	EV-A71. Strain BrCr	3x LoD
Enterovirus B	ZeptoMetrix (0810019CF) <sup>a</sup>	Coxsackievirus B5	1x LoD
	ZeptoMetrix (0810017CF) <sup>a</sup>	Coxsackievirus A9, species B	1x LoD
	ATCC (VR-28)	Species B, Serotype CV-B1, Strain Conn-5	3x LoD
	ATCC (VR-29)	Species B, Serotype CV-B2. Strain Ohio-1	3x LoD
	ZeptoMetrix (0810075CF)	Coxsackievirus B4	1:100 from stock
	ZeptoMetrix (0810076CF)	Echovirus 6	1:100 from stock
	ZeptoMetrix (0810077CF)	Echovirus 9	1:100 from stock
	ZeptoMetrix (0810074CF)	Coxsackievirus B3	1:10000 from stock
	NCPV (0901047v)	Echovirus 18	3x LoD
	ATCC (VR-41) <sup>c</sup>	Species B, Serotype E-11	3x LoD <sup>c</sup>
Enterovirus C	ATCC (VR-1023) <sup>a</sup>	Coxsackievirus A17, species C. Strain G-12	1x LoD
	ATCC (VR-583) <sup>a</sup>	Coxsackievirus A24. Strain DN-19	1x LoD
	ATCC (VR-850) <sup>c</sup>	Coxsackievirus A21. Strain Kuykendall [V-024-001-012]	3x LoD <sup>c</sup>
	ATCC (VR-169)	A11 – Belgium-1	3x LoD
	ATCC (VR-1488)	A13 – Flores	3x LoD
	ATCC (VR-182)	A22 – Chulman	1:100 from stock
	ATCC (VR-178)	A20 – IH Pool 35	1:100 from stock
	ATCC (VR-176)	A18 – G-13	1:100 from stock
	NCTC (0812075v)	CV-A21. Strain H06452 472	3x LoD
	NCTC (0812074v)	CV-A21. Strain H06418 508	0.03x LoD
Enterovirus D	ATCC (VR-836) <sup>a</sup>	EV 70, species D, strain J670/71	1x LoD
	ATCC (VR-1823) <sup>a</sup>	Enterovirus D68. Strain US/MO/14-18947	1x LoD
	ZeptoMetrix (0810237CF)	Enterovirus 68. 2007 Isolate	0.03x LoD
	ATCC (VR-1824)	Enterovirus D68. Strain US/IL/14-18952	3x LoD
	ATCC (VR-1197)	D68. Strain F02-3607 Corn	3x LoD
	ZeptoMetrix (0810302CF)	Type 68 Major Group (09/2014 Isolate 2)	1:100 from stock
	ATCC (VR-1825)	Enterovirus D68. Strain US/KY/14-18953	3x LoD
	ATCC (VR-1826)	Enterovirus D68. Strain Fermon	3x LoD
	BEI Resources (NR- 49130)	Enterovirus D68. US/MO/14- 18949	3x LoD
	BEI Resources (NR- 51998)	Enterovirus D68. USA/2018-23089	3x LoD
	ATCC (MYA-4567) <sup>a</sup>	Serotype D strain WM629, type VNIV	1x LoD

<i>Cryptococcus neoformans</i>	ATCC (208821) <sup>a</sup>	H99	1x LoD
	ATCC (32045)	type strain, CBS 132	3x LoD
	ATCC (MYA-4564)	Serotype A strain WM148, type VNI	1:1000 from stock
	ATCC (13690)	M2092	1:100 from stock
	ATCC (MYA-4566)	Serotype AD strain WM628, type VNIII	1:1000 from stock
	ZeptoMetrix (0801803)	Serotype A	3x LoD
	BEI Resources (NR- 50335)	NIH9hi90	1:100 from stock
	BEI Resources (NR- 50332)	NIH306	1:100 from stock
	BEI Resources (NR- 48776)	Var grubiiYL99 $\alpha$	1:100 from stock
<i>Cryptococcus gattii</i>	ATCC (MYA-4094) <sup>a</sup>	Serotype B strain R272, type VGIIb	1x LoD
	ATCC (MYA-4877) <sup>a</sup>	A6MR38	1x LoD
	ATCC (MYA-4560)	Serotype B strain WM179, type VGI	1:100 from stock
	ATCC (MYA-4562)	Serotype B strain WM161, type VGIII	1:1000 from stock
	ATCC (MYA-4563)	Serotype C strain WM779, type VGIV	1:1000 from stock
	ATCC (MYA-4138)	A1M R265	3x LoD
	ATCC (14248)	110 [CBS 883]	1:1000 from stock
	BEI Resources (NR- 50184)	AIR265	1:100 from stock
	BEI Resources (NR- 50195)	Alg166	1:100 from stock
	BEI Resources (NR- 50198)	Alg254	1:100 from stock

<sup>a</sup> Strains tested and evaluated during the Limit of Detection studies.

<sup>b</sup> Commercial artificial dsDNA fragment (gBlock) including *ctrA* gene sequence was tested due to unavailability of analytical sample for this variant (GenBank Accession ID HQ156899).

<sup>c</sup> Higher concentration tested to meet 100% detection rate (30xLoD for ATCC (VR-1550, VR-850 and VR-41) and 3xLoD for ZeptoMetrix (0804339)).

125 out of 130 pathogen strains were successfully detected by the QIAstat-Dx ME Panel when tested. Five strains were not detected by the assay (Table 2).

**Table 2: Inclusivity Strains Not Detected by the QIAstat-Dx ME Panel**

Target	Strain/Serotype
<i>Escherichia coli</i> K1	NCDC Bi 7509-41 Serotype O7:K1(L):NM
<i>Escherichia coli</i> K1	Z136 CTX-M-15
Enterovirus C	CV-A21 Strain H06452 472
Enterovirus C	CV-A21, Strain H06418 508
<i>Streptococcus agalactiae</i>	Serotype III. Typing strain D136C(3) [3 Cole 106, CIP 52.45]

To make assay reactivity predictions of all primers-probe oligonucleotide sequences included in the panel against publicly available sequence databases to detect any possible cross-reaction or unexpected detection of any primer set, in silico analysis was performed. In addition, strains not available for in vitro testing were included in in silico analysis to confirm the predicted inclusivity of the different strains of the same organisms (Table 3).

**Table 3: Clinically Relevant Strains/Serotypes Detected per Pathogen**

Target	Clinically relevant strains/subtypes detected
<i>Neisseria meningitidis</i> (encapsulated)	All the encapsulated serotypes (A, B, C, D, E, H, I, K, L, NG, W, W135, X, Y, Z, 29E)
<i>Cryptococcus gattii/neoformans</i>	All <i>Cryptococcus</i> spp. serotypes: Serotype A ( <i>C. neoformans</i> var <i>neoformans</i> ), serotype D ( <i>C. neoformans</i> var <i>grubii</i> ), serotypes B and C ( <i>C. gattii</i> including all VGI, VGII, VGIII, VGIV molecular types)
<i>Listeria monocytogenes</i>	Serotypes 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 4e, 7
<i>Haemophilus influenzae</i>	All encapsulated serotypes (a, b, c, d, e, f) and unencapsulated strains (nontypeable, NTHi) including var. <i>H. aegyptus</i>
Enterovirus	Coxsackievirus A (CV-A1 through CV-A24), coxsackievirus B (CV-B1 through CV-B6), Echovirus (E-1 through E-33), Enterovirus A (EV-A71, EV-A76, EV-A89 through EV-A92, EV-A119, EVA120), Enterovirus B (EV-B69, EV-B73 through EV-B75, EV-B79, EV-B80 through EV-B88, EVB93, EV-B97, EV-B98, EV-B100, EV-B101, EV-C99, EV-C102, EV-C104, EV-C105, EV-C109, EV-C116 through EV-C118), Enterovirus EV-B106, EV-B107, EV-B111), Enterovirus C (EVC96, D (EV-D68, EV-D70, EV-D94), Poliovirus (PV-1 through PV-3)
<i>Escherichia coli</i> K1	K1 strains (excluding general <i>E.coli</i> strains)
Rest of On-Panel organism with no biological subclassification ( <i>S. pneumoniae</i> , <i>S. agalactiae</i> , <i>S. pyogenes</i> )	All genomic sequences available in databases detected

## 2. Precision/Reproducibility:

### a. Multi-site Reproducibility Study

Reproducibility of the QIAstat-Dx ME Panel assay was evaluated at three different sites (one internal, two external) using three blinded panels (Table 4) representing a subset of targets detected by the assay. The study evaluated positive samples (artificial CSF spiked with selected pathogens) containing analytes at both 1X LoD and 3X LoD concentrations as well as negative samples (artificial CSF). Testing occurred over 5 non-consecutive days with each sample evaluated in two replicates/site/day. The study also included 13 operators across the three study sites and a total of 4 cartridge lots.

A total of 90 replicates per target were tested at each concentration evaluated. Samples were prepared in bulk, divided into single-use aliquots, and stored frozen at -80°C or below until usage. Samples were evaluated for expected reactivity before inclusion in the study.

**Table 4: Reproducibility Study Panels**

Mix	Pathogen	Source	Catalog ID	Lot	Tested concentration
1	<i>Cryptococcus gattii</i>	ATCC	MYA-4094	7730064	3x LoD
	<i>Streptococcus agalactiae</i>	ATCC	13813	70011789	
	<i>Listeria monocytogenes</i>	ATCC	19115	70029351	
	Enterovirus A	ATCC	VR-1801	70002938	
	<i>E. coli</i> K1	ATCC	700973	70022532	
2	<i>Cryptococcus gattii</i>	ATCC	MYA-4094	7730064	1x LoD
	<i>Streptococcus agalactiae</i>	ATCC	13813	70011789	
	<i>Listeria monocytogenes</i>	ATCC	19115	70029351	
	Enterovirus A	ATCC	VR-1801	70002938	
	<i>E. coli</i> K1	ATCC	700973	70022532	
3	Negative (aCSF)	Ecocyte Bioscience	LRE-S-	aCSF A:	N/A
			LSG-1000-1	5002764104	
				aCSF B:	
				181222A	

Reproducibility was assessed by evaluating the agreement of positive or negative results from the investigational assay with the expected results per for each target and concentration evaluated. The study acceptance criteria for each panel member were the following:

- 3x LoD: the observed proportion of positive calls must be  $\geq 96.7\%$  (87 / 90) (95% CI: 90.6%-99.3%).
- 1x LoD: the observed proportion of positive calls must be  $\geq 90.0\%$  (81 / 90) (95% CI: 81.9%-95.3%).
- Negative: The proportion of negative results must be  $\geq 96.7\%$  (87 / 90) (95% CI: 90.6%-99.3%).

Raw data interpretation and results reporting were automatically done by the Operational Module Application Software and the Assay Definition File (ADF) that is installed in the QIAstat-Dx Analyzer 1.0 instrument.

At 3x LoD concentration, all targets gave 100% correct calls. At 1x LoD concentration, all targets gave 100% correct calls, except for *Listeria monocytogenes* (Table 5). All negative samples returned a negative call 100% of the time. The study results were acceptable and demonstrate appropriate reproducibility of the assay.

**Table 5: Reproducibility Study Results Summary**

Target	Concentration	Agreement with Expected Results			
		Site 1	Site 2	Site 3	All Sites [95% CI]
Bateria					
Escherichia Coli K1	3x LoD 1044 CFU/mL	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90) [95.9%-100.0%]
	1x LoD 348 CFU/mL	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90) [95.9%-100.0%]
Listeria monocytogenes	3x LoD 19920 CFU/mL	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90) [95.9%-100.0%]
	1x LoD 6640 CFU/mL	96.7% (29/30)	100.0% (30/30)	100.0% (30/30)	98.9% (89/90) [94.0%-99.8%]
Streptococcus agalactiae	3x LoD 10140 CFU/mL	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90) [95.9%-100.0%]
	1x LoD 3380 CFU/mL	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90) [95.9%-100.0%]
Virus					
Enterovirus (EV)	3x LoD 480 CFU/mL	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90) [95.9%-100.0%]
	1x LoD 160 CFU/mL	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90) [95.9%-100.0%]

Yeast					
<i>Cryptococcus gattii</i> / <i>Cryptococcus neoformans</i> *	3x LoD 39600 CFU/mL	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90) [95.9%-100.0%]
	1x LoD 13200 CFU/mL	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90) [95.9%-100.0%]
Negative (no analyte)					
Negative CSF Matrix		100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90) [95.9%-100.0%]

\**Cryptococcus gattii* and *Cryptococcus neoformans* are not differentiated.

3. Linearity:

Not applicable. The device is a qualitative assay.

4. Analytical Specificity/Interference:

a. Analytical Specificity (Cross-Reactivity)

The analytical specificity of the assay was evaluated using both *in vitro* wet-testing and *in silico* analysis to assess the potential cross-reactivity and exclusivity of the QIAstat-Dx ME Panel. On-panel organisms were tested to assess the potential for intra-panel cross-reactivity and off-panel organisms were tested to evaluate cross-reactivity with organisms not covered by the panel (panel exclusivity). Off-panel organisms were selected due to being known to colonize the central nervous system or cause meningitis and/or encephalitis symptoms, are common skin flora or laboratory contaminants, are genetically similar to on-panel analytes, or are microorganisms for which much of the population may have been infected.

Samples were prepared by spiking potential cross-reactive organisms (Table 6) into artificial CSF matrix (see Section B.2. for matrix comparison) at  $10^5$  TCID<sub>50</sub>/mL for viral targets,  $10^6$  CFU/mL for fungi/yeast target, and  $10^6$  CFU/mL for bacterial and fungi/yeast target, or the highest concentration possible based on the organism stock.

All on-panel pathogens evaluated in the study resulted in specific detection, and all off-panel pathogens tested showed a negative result with no cross-reactivity was observed except for the pathogens shown in Table 6 below.

**Table 6: Cross-Reactivity Organisms Evaluated**

Type	Pathogen	Strain/Serotype	Pathogen	Strain/Serotype
Bacteria	<i>Bacillus cereus</i>	Z091	<i>Proteus mirabilis</i>	LRA 08 01 73 [API SA, DSM 6674]
	<i>Citrobacter freundii</i>	[ATCC 13316, NCTC 9750]	<i>Pseudomonas aeruginosa</i>	PRD-10 [CIP 103467, NCIB 10421, PCI 812]
	<i>Corynebacterium striatum</i>	CDC F6683	<i>Salmonella bongori</i>	CIP 82.33
	<i>Corynebacterium urealyticus</i>	3 [Garcia strain]	<i>Salmonella enterica</i>	CDC K-1891 [ATCC 25928]

	<i>Cronobacter (Enterobacter) sakazakii</i>	CDC 4562-70	<i>Serratia marcescens</i>	PCI 1107
	<i>Enterobacter aerogenes</i>	Z052	<i>Shigella boydii</i>	CDC C-123
	<i>Enterobacter cloacae</i>	CDC 442-68	<i>Shigella flexneri</i>	Z046
	<i>Escherichia coli (non-K1)</i>	2003-3055	<i>Shigella sonnei</i>	AMC 43-GG9
	<i>Escherichia fergusonii</i>	Z302	<i>Staphylococcus aureus</i>	FDA 209
	<i>Escherichia hermannii</i>	CDC 980-72	<i>Staphylococcus capitis</i>	PRA 360 677
	<i>Escherichia vulneris</i>	CDC 875-72	<i>Staphylococcus epidermidis</i>	FDA strain PCI 1200
	<i>Haemophilus ducreyi DCC1476</i>	[Sweden 15A-25]	<i>Staphylococcus haemolyticus</i>	SM 131
	<i>Haemophilus haemolyticus</i>	NCTC 10659	<i>Staphylococcus hominis</i>	Z031
	<i>Haemophilus parahaemolyticus</i>	536 [NCTC 8479]	<i>Staphylococcus lugdunensis</i>	LRA 260.05.79
	<i>Haemophilus parainfluenzae</i>	NCTC 7857	<i>Staphylococcus saprophyticus</i>	NCTC 7292
	<i>Klebsiella pneumoniae</i>	NCTC 9633 [NCDC 298-53, NCDC 410-68]	<i>Streptococcus anginosus</i>	NCTC 10713
	<i>Listeria innocua</i>	SLCC 3379	<i>Streptococcus bovis</i>	Z167
	<i>Listeria ivanovii</i>	Li 1979	<i>Streptococcus dysgalactiae</i>	Grouping strain C74
	<i>Morganella morganii</i>	AM-15	<i>Streptococcus intermedius</i>	Z126
	<i>Mycoplasma genitalium</i>	M30	<i>Streptococcus mitis</i>	(tigrinus) Clinical Isolate
	<i>Neisseria gonorrhoeae</i>	Z017	<i>Streptococcus mutans</i>	LRA 28 02 81
	<i>Neisseria lactamica</i>	NCDC A7515	<i>Streptococcus oralis</i>	Z307
	<i>Neisseria mucosa</i>	AmMS 138	<i>Streptococcus pseudopneumoniae</i>	CDC-SS-1757
	<i>Neisseria sicca</i>	AMC 14-D-1	<i>Streptococcus salivarius</i>	C699
	<i>Pantoea agglomerans</i>	Enterobacter agglomerans	<i>Streptococcus sanguinis</i>	DSS-10
	<i>Propionibacterium acnes</i>	NCTC 737		
Fungi/parasite	<i>Aspergillus fumigatus</i>	Z014	<i>Cryptococcus adeliensis</i> <i>Cryptococcus adeliae</i> <i>Naganishia adelienses</i>	<i>Cryptococcus adeliae</i>
	<i>Candida albicans</i>	CBS 562	<i>Cryptococcus albidus</i>	AmMS 228
	<i>Candida dubliniensis</i>	Z145	<i>Cryptococcus amyloletus</i>	NRRY Y-7784
	<i>Candida glabrata</i>	CBS 138	<i>Cryptococcus depauperatus</i> <i>Aspergillus depauperatus</i> <i>Filobasidiella depauperate</i>	K [ARSEF 2058, CBS 7842]
	<i>Candida krusei</i>	N/A	<i>Cryptococcus flavescens</i> <i>Papiliotrema flavescens</i>	<i>Cryptococcus alurentii</i> var. Flavescens (Saito) Lodder et Kerger-van Rij
	<i>Candida lusitanae</i>	Z010	<i>Cryptococcus laurentii</i>	CBS 139

	<i>Candida metapsilosis</i>	MCO429	<i>Cryptococcus uniguttulatus</i>	AmMS 234
	<i>Candida orthopsilosis</i>	MCO471	<i>Cryptococcus wingfieldii</i> <i>Tsuchiyaea wingfieldii</i>	OTU 26
	<i>Candida parapsilosis</i>	CBS 604	<i>Filobasidium capsuligenum</i>	ML-186
	<i>Candida tropicalis</i>	Vitek #8935	<i>Naegleria fowleri</i>	Genomic DNA from <i>Naegleria fowleri</i>
	<i>Candida viswanathii</i>	PK 233 [NCYC 997, pK233]	<i>Toxoplasma gondii</i>	Haplogroup 2
Viruses	<i>Adenovirus A12</i>	Huie	Human Rhinovirus A16	11757
	Adenovirus C2	Adenoid 6 (NIAID 202-001-014)	Human Rhinovirus A1b	2060
	Adenovirus D20	A.A	Human Rhinovirus B3	FEB
	Adenovirus E4	RI-67	Human Rhinovirus B83	Barlor 7 [V-190-001-021]
	Adenovirus F41	Tak	Influenza A H1N1	A/Florida/3/2006
	BK polyoma virus	N/A	Influenza A H1N1-2009	A/California/08/2009
	Coronavirus 229E	229E	Influenza A H3N2	A/Port Chalmers/1/73
	Coronavirus NL63	NL63 (Amsterdam I)	Influenza B	B/Virginia/ATCC4/2009
	Coronavirus OC43	OC43	JC polyoma virus	MAD-4
	Cytomegalovirus	Davis	Measles Virus	Edmonston
	Dengue virus (Type 2)	New Guinea C	Mumps Virus	Jones
	Epstein-Barr Virus	B95-8	Parainfluenza virus 2	Greer
	Hepatitis B virus (HBV)	N/A	Parainfluenza virus 4	N/A
	Hepatitis C virus (HCV)	N/A	Parvovirus B19	B19
	Herpes simplex virus 1	Macintyre	Respiratory Syncytial Virus	A2
	Herpes simplex virus 2	HSV-2. (Strain: MS)	Rotavirus RRV	(Rhesus Rotavirus)
	Human herpes virus 6	HHV-6B. (Strain: Z29)	Rubella Virus	N/A
	Human herpes virus 7	SB	St. Louis Encephalitis Virus	Parton
	Human herpes virus 8	N/A	Varicella-zoster virus	Ellen
	Human Immunodeficiency	Quatitative Synthetic Human Immunodeficiency Virus (HIV-1) RNA	West Nile Virus	1986
	Human parechovirus	Serotype 3		
Yeast			<i>Saccharomyces cerevisiae</i>	NRRL Y-567

**Table 7: Cross-Reactive Pathogens Summary**

QIAstat-Dx ME Panel Target	Potential Cross-Reactive Organism	Cross Reactive Concentration
<i>Haemophilus influenzae</i>	<i>Haemophilus haemolyticus</i>	$\geq 1.00\text{E}+03$ CFU/mL
<i>Cryptococcus neoformans/gattii</i>	<i>Cryptococcus wingfieldii</i> <i>Tsuchiyaea wingfieldii</i>	$\geq 1.00\text{E}+01$ CFU/mL
	<i>Cryptococcus flavescens</i> <i>Papiliotrema flavescens</i>	$\geq 4.00\text{E}+03$ CFU/mL
<i>Cryptococcus neoformans/gattii</i>	<i>Cryptococcus amyloletus</i>	$\geq 1.00\text{E}+01$ CFU/mL

*In silico* analysis was also performed for the QIAstat-Dx ME Panel primer/probe designs in two steps to further characterize the sequence specificity of the RT-PCR assays to detect possible unspecific homologies and/or unspecific cross-reactions.

The result of the analysis identified six potential cross-reactive off-panel targets (Table 8).

**Table 8: *In silico* Cross-Reactivity Analysis Summary**

Off-Panel Organism	On-Panel Signal
<i>Streptococcus pseudopneumoniae</i> *	<i>Streptococcus pneumoniae</i>
<i>Listeria innocua</i> *	<i>Listeria monocytogenes</i>
<i>Cryptococcus amyloletus</i>	<i>Cryptococcus neoformans/gattii</i>
<i>Cryptococcus depauperatus</i> *	
<i>Cryptococcus wingfieldii</i>	

\*Potential cross-reactivity was not confirmed by *in vitro* wet-testing.

b. Interfering Substances:

An interference study was conducted to evaluate whether assay performance was affected by commonly encountered interfering substances (Table 9). The substances tested in the study (21) included endogenous as well as exogenous substances that are commonly found and/or introduced into CSF specimens during collection.

All QIAstat-Dx ME Panel target organisms were tested at 3x LoD in artificial CSF matrix and testing was performed in triplicate. Potential interfering substances were spiked into the samples at high concentrations intended to reflect worst case concentrations encountered in clinical samples.

**Table 9: Interference Testing Results Summary**

	Name	Concentration Tested	Interference Observed?
Endogen	Human blood	10% (v/v)	No
	gDNA	20 µg/mL	No
	D(+)Glucose	10 mg/mL	No
	L-lactate (Na)	2.2 mg/mL	No

	Immunoglobulin G (human)	20 mg/mL	No
	Albumin (human)	30 mg/mL	No
	Peripheral blood mononuclear cells	10,000 cells/ $\mu$ L	No
Exogenous	Chlorhexidine	0.4% (w/v)	No
	Ethanol	7% (v/v)	No
	Bleach	1% (v/v)	Yes
	Bleach	0.1% (v/v)	Yes
	Bleach	0.01% (v/v)	No
	Acyclovir	69 $\mu$ g/mL	No
	Amphotericin B	5.1 $\mu$ g/mL	No
	Ampicillin	210 $\mu$ g/mL	No
	Ceftriaxone	840 $\mu$ g/mL	No
	Cefotaxime	645 $\mu$ g/mL	No
	Ganciclovir	25 $\mu$ g/mL	No
	Gentamicin	30 $\mu$ g/mL	No
	Meropenem	339 $\mu$ g/mL	No
	Vancomycin	180 $\mu$ g/mL	No
	Voriconazole	11 $\mu$ g/mL	No
	Oseltamivir	0.399 $\mu$ g/mL	No

Most evaluated endogenous and exogenous substances have been confirmed not to interfere with any of the panel target assays at concentrations potentially found in clinical samples. However, interference was observed for Bleach present at concentrations above 0.01%.

c. Microbial Interference/Competitive Inhibition:

A study was conducted to evaluate whether high concentrations of viruses ( $10^5$  units/mL) or bacteria ( $10^6$  CFU/mL) could impact assay performance. Briefly, non-target organisms (Table 10) were individually mixed with artificial CSF matrix containing one of the four spiked targeted QIAstat Dx ME Panel organism mixes (3x LoD) (Table 11). Testing was performed in triplicate.

**Table 10: Microbial Interference Organisms Tested**

Name	Concentration Tested
Epstein-Barr virus	$10^5$ cp/mL
Influenza A H1N1-2009	$10^5$ CEID <sub>50</sub> /mL
<i>Cutibacterium acnes</i>	$10^6$ CFU/mL
<i>Staphylococcus epidermidis</i>	$10^6$ CFU/mL
<i>Escherichia coli</i> (non-K1)	$10^6$ CFU/mL
<i>Staphylococcus aureus</i>	$10^6$ CFU/mL
Measles virus	$10^5$ TCID <sub>50</sub> /mL

**Table 11: On Panel Organisms Evaluated in Microbial Interference Testing**

Mix	Pathogen	Tested concentration
1	<i>Cryptococcus neoformans/gattii</i>	
	<i>Streptococcus agalactiae</i>	
	<i>Listeria monocytogenes</i>	
	Enterovirus A	
2	<i>Streptococcus pneumoniae</i>	3x LoD
	<i>Neisseria meningitidis</i>	
	<i>Haemophilus influenzae</i>	
	<i>Escherichia coli</i> K1	
3	<i>Streptococcus pyogenes</i>	3x LoD
4	Negative (CSF)	N/A

All QIAstat-Dx ME Panel organisms were successfully detected when tested in combination with the potential microbial interferents.

5. Assay Reportable Range:

Not applicable. The device is a qualitative assay.

6. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

a. Assay Controls

i. Internal Controls:

The QIAstat-Dx ME Panel includes an Internal Control, which is titrated *Schizosaccharomyces pombe*, a yeast (fungi) that is included in the cartridge in dried form and is rehydrated upon sample loading. This Internal Control material verifies all steps of the analysis process, including sample homogenization, lysis of viral and cellular structures (by means of chemical and mechanical disruption), nucleic acid purification, reverse transcription, and real-time PCR.

A positive signal for the Internal Control indicates that all processing steps performed by the QIAstat-Dx ME Panel were successful.

A negative signal of the Internal Control does not negate any positive results for detected and identified targets, but it does invalidate all negative results in the analysis. Therefore, the test should be repeated if the Internal Control signal is negative.

ii. Recommended External Control:

All external quality control testing should be performed in accordance with local, state, and federal regulations or accreditation organizations and should follow the laboratory standard quality control procedures.

Control materials are not provided with the QIAstat-Dx ME Panel.

b. Sample Stability

A sample stability study was conducted to demonstrate that clinical cerebrospinal fluid specimens may be stored at room temperature (15 to 25°C) for up to twenty-four hours, at refrigerated conditions (2 to 8°C) for up to seven days or in the freezer (-15 to -25°C) and (-60°C to -90°C) for up to two and four months, respectively, before testing with the QIAstat-Dx ME Panel without affecting the performance.

Positive samples used in this study were prepared in clinical negative CSF matrix spiked with targets at 2.5x LoD concentration (Table 12). At least three small pools of clinical matrix were used for sample preparation. Pathogen-negative samples without added pathogens were not assessed in this study since negative results from the non-spiked targets have been analyzed. Samples were prepared in bulk and divided into single aliquots that were tested after specific storage conditions.

**Table 12: Sample Stability Study Organism Panels**

Mix	Target	Supplier	Catalog ID
1	<i>Cryptococcus gattii</i>	ATCC	MYA-4877
	<i>Streptococcus agalactiae</i>	ATCC	13813
	<i>Listeria monocytogenes</i>	ZeptoMetrix	801534
2	<i>Streptococcus pneumoniae</i>	ATCC	33400
	<i>Neisseria meningitidis</i> (encapsulated)	ATCC	13090.00
	<i>Haemophilus influenzae</i>	ATCC	10211
	<i>Escherichia coli</i> K1	ATCC	700973.000
3	<i>Streptococcus pyogenes</i>	ZeptoMetrix	804351
4	Enterovirus A	ZeptoMetrix	0810107CF

Right after sample preparation, at least twenty replicates of each sample were tested as a referenced condition (T0). The rest of the prepared aliquots were then stored and tested.

Each storage condition was considered suitable for storage if all spiked pathogens at 2.5x LoD generated a detection rate of  $\geq 95\%$  ( $\geq 19/20$ ) of the tested aliquots per storage condition and, additionally, all pathogens not included in the respective sample resulted in a negative signal.

All panel analytes included in Sample Mixes 1, 2 and 3 passed the study acceptance criteria indicating a true positive agreement of at least 95% for all time points assessed except for *Neisseria meningitidis* (ATCC; 13090) in Sample Mix 2, which showed a detection rate  $< 95\%$  (18/20) at 121 days (-60°C to -90°C). An investigation was conducted to assess the Ct drift of *Neisseria meningitidis* (ATCC; 13090) across the different timepoints (including T0) for the storage condition -60°C to -90°C. Results of this analysis showed that the regression line for this target was well within the allowable drift limit and was deemed to be statistically stable at all the timepoints assessed at the storage condition -60°C to -90°C. Therefore, *Neisseria meningitidis* test results at 121 days (-60°C to -90°C) were accepted.

A detection rate of <95% was observed for Enterovirus at 31 days. Investigation concluded cartridges used for Enterovirus exceeded their shelf life. Results obtained during subsequent timepoints passed acceptance criteria.

Data from the study was adequate to support the storage claims included in the labeling. The results of this study support claims for storage of cerebrospinal fluid for 24 hours at 15-25°C or refrigerated for up to seven days at 2-8°C prior to testing samples with the QIAstat-Dx ME Panel. No claim for use of frozen specimens was included in the Package Labeling.

c. Freeze/Thaw Study

A fresh vs frozen study was performed to demonstrate equivalent assay performance when samples are frozen at -60°C to -90°C for at least 12 hours and subjected to 3 freeze-thaw cycles prior testing and to support the use of frozen samples during the validation studies.

For the fresh vs. frozen study, analytical samples were manufactured using organisms from commercial suppliers (Table 15). Samples consisted of mixtures of organisms spiked into pre-screened negative clinical CSF matrix at a final concentration of 5x LoD, 1x LoD and 0.1x LoD as defined by the Limit of Detection in Combined Samples.

Sample mixes were prepared in pools, divided into aliquots, and either immediately tested (fresh) or frozen at -60°C to -90°C for at least 12 hours. Samples were frozen twice with at least two hours between freeze/thaw cycles and then thawed again before testing at each timepoint with the QIAstat-Dx ME Panel for a total of 3 freeze/thaw cycles. Testing was conducted by a minimum of one operator using at least one lot of QIAstat-Dx ME Panel on 3 or more QIAstat-Dx Analyzer 1.0 instruments and replicated as described in Table 13.

**Table 13: Number of Replicates Tested and Expected Outcome of Fresh vs Frozen Study**

Concentration	Minimum Number of Replicates per Strain per Storage Condition	Expected Results (% positive)
5x	10	100
1x	30	≥95
0.1x	10	>0
Negative	10	0

The results (Table 14) of the freeze/thaw study support sample stability through three freeze/thaw cycles prior to testing with the QIAstat-Dx ME Panel.

**Table 14: Freeze/Thaw Study Results Summary**

Target	Supplier	Catalog ID	Concentration	Percent Agreement Fresh	Percent Agreement Frozen
<i>Streptococcus agalactiae</i>	ZeptoMetrix	801545	5x LoD	100.0%	100.0%
			1x LoD	100.0%	100.0%
			0.1x LoD	70.0%	80.0%

	ATCC	13813	5x LoD	100.0%	100.0%
			1x LoD	100.0%	100.0%
			0.1x LoD	100.0%	100.0%
<i>Listeria monocytogenes</i>	ZeptoMetrix	801534	5x LoD	100.0%	100.0%
			1x LoD	100.0%	100.0%
			0.1x LoD	80.0%	80.0%
	ATCC	19115	5x LoD	100.0%	100.0%
			1x LoD	100.0%	100.0%
			0.1x LoD	70.0%	60.0%
<i>Streptococcus pneumoniae</i>	ZeptoMetrix	801439	5x LoD	100.0%	100.0%
			1x LoD	96.7%	100.0%
			0.1x LoD	30.0%	50.0%
	ATCC	33400	5x LoD	100.0%	100.0%
			1x LoD	100.0%	100.0%
			0.1x LoD	9.00%	80.0%
<i>Neisseria meningitidis</i> (encapsulated)	ATCC	13090	5x LoD	100.0%	100.0%
			1x LoD	96.7%	100.0%
			0.1x LoD	50.0%	90.9%
	ATCC	35561	5x LoD	100.0%	100.0%
			1x LoD	100.0%	100.0%
			0.1x LoD	100.0%	90.0%
<i>Haemophilus influenzae</i>	ATCC	10211	5x LoD	100.0%	100.0%
			1x LoD	96.7%	100.0%
			0.1x LoD	100.0%	100.0%
	ATCC	8142	5x LoD	100.0%	100.0%
			1x LoD	100.0%	100.0%
			0.1x LoD	100.0%	100.0%
<i>Escherichia coli</i> K1	ATCC	700973	5x LoD	100.0%	100.0%
			1x LoD	100.0%	100.0%
			0.1x LoD	60.0%	90.9%
	ATCC	11775	5x LoD	100.0%	100.0%
			1x LoD	100.0%	100.0%
			0.1x LoD	70.0%	90.0%
<i>Streptococcus pyogenes</i>	ZeptoMetrix	804351	5x LoD	100.0%	100.0%
			1x LoD	100.0%	100.0%
			0.1x LoD	20.0%	40.0%
	ATCC	19615	5x LoD	100.0%	100.0%
			1x LoD	100.0%	100.0%
			0.1x LoD	100.0%	100.0%
Enterovirus A	ZeptoMetrix	0810107CF	5x LoD	100.0%	100.0%

			1x LoD	100.0%	100.0%
			0.1x LoD	40.0%	50.0%
	ATCC	VR-1801	5x LoD	100.0%	100.0%
			1x LoD	86.7%	83.3%
			0.1x LoD	20.0%	50.0%
<i>Cryptococcus neoformans</i>	ATCC	MYA-4567	5x LoD	100.0%	100.0%
			1x LoD	96.7%	100.0%
			0.1x LoD	40.0%	40.0%
	ATCC	208821	5x LoD	100.0%	100.0%
			1x LoD	100.0%	100.0%
			0.1x LoD	90.0%	100.0%
Negative CSF Matrix				100.0%	100.0%

#### Carryover

A carryover study was performed to evaluate the potential occurrence of cross-contamination between consecutive runs when using the QIAstat-Dx ME Panel on the QIAstat-Dx Analyzer 1.0. Alternating CSF samples containing either high-positive ( $10^5$ – $10^6$  organism/mL) or no organisms were evaluated on two QIAstat-Dx Analyzer 1.0 instruments.

No carryover between samples was observed in the QIAstat-Dx ME Panel, demonstrating that the system design and recommended sample handling and testing practices are effective in preventing unexpected results due to carryover or cross-contamination between samples.

#### 7. Detection Limit:

##### Individual Limit of Detection (LoD)

The LoD was defined as the lowest concentration at which  $\geq 95\%$  of samples generate a positive result. Dilutions of 26 commercial pathogen strains (Table 15) were prepared using artificial cerebrospinal fluid. Equivalence between the artificial CSF matrix and native CSF matrix was established in a separate matrix equivalency study (see Section B.2).

**Table 15: Individual Spiked Organism Limit of Detection Results**

Target	Supplier	Catalog ID	Individual LoD	Detection Rate
<b>Bacteria</b>				
<i>Escherichia coli</i> K1	ATCC	700973	348 CFU/mL	30/30
<i>Escherichia coli</i> K1	ATCC	11775	786 CFU/mL	30/30
<i>Haemophilus influenzae</i>	ATCC	10211	316 CFU/mL	32/32
<i>Haemophilus influenzae</i>	ATCC	8142	2540 CFU/mL	30/30
<i>Listeria monocytogenes</i>	ZeptoMetrix	801534	1860 CFU/mL	21/21

<i>Listeria monocytogenes</i>	ATCC	19115	6640 CFU/mL	30/30
<i>Neisseria meningitidis</i> (encapsulated)	ATCC	13090	0.0828 CFU/mL	31/32
<i>Neisseria meningitidis</i> (encapsulated)	ATCC	35561	13.3 CFU/mL	30/30
<i>Streptococcus agalactiae</i>	ZeptoMetrix	801545	1750 CFU/mL	30/30
<i>Streptococcus agalactiae</i>	ATCC	13813	3380 CFU/mL	31/31
<i>Streptococcus pneumoniae</i>	ZeptoMetrix	801439	714 CFU/mL	29/30
<i>Streptococcus pneumoniae</i>	ATCC	33400	0.622 CFU/mL	29/29
<i>Streptococcus pyogenes</i>	ZeptoMetrix	804351	1800 CFU/mL	30/30
<i>Streptococcus pyogenes</i>	ATCC	19615	91 CFU/mL	30/30
<b>Viruses</b>				
Enterovirus A	ZeptoMetrix	0810107CF	3.79 TCID <sub>50</sub> /mL	31/31
Enterovirus A	ATCC	VR-1801	160 TCID <sub>50</sub> /mL	30/30
Enterovirus B	ZeptoMetrix	0810019CF	89.1 TCID <sub>50</sub> /mL	30/30
Enterovirus B	ZeptoMetrix	0810017CF	43.6 TCID <sub>50</sub> /mL	28/29
Enterovirus C	ATCC	VR-1023	15.8 TCID <sub>50</sub> /mL	30/30
Enterovirus C	ATCC	VR-583	4.99 TCID <sub>50</sub> /mL	30/30
Enterovirus D	ATCC	VR-836	49.9 TCID <sub>50</sub> /mL	30/31
Enterovirus D	ATCC	MYA-4567	506 TCID <sub>50</sub> /mL	30/30
<b>Yeast</b>				
<i>Cryptococcus neoformans</i>	ATCC	MYA-4567	2210 CFU/mL	31/31
<i>Cryptococcus neoformans</i>	ATCC	208821	164 CFU/mL	31/31
<i>Cryptococcus gattii</i>	ATCC	MYA-4094	13200 CFU/mL	30/30
<i>Cryptococcus gattii</i>	ATCC	MYA-4877	2600 CFU/mL	29/29

i. Multi-Spiked Limit of Detection (LoD)

The LoD for each pathogen was assessed in multiple-spiked samples containing up to five organisms to determine if multiple analytes in a single CSF sample during the analytical studies for potential loss of detection when multiple targets are present in clinical specimens (co-infections). Multiplexed samples were tested at 5x LoD, 1x LoD, and 0.1x LoD concentrations following the LoDs established in the individual LoD Study.

Samples consisting of three different organism mixes with up to five targeted organisms each were compared to contrived single-spike samples for the following representative panel analytes: bacterium (*E. coli* K1), yeast (*C. neoformans*), DNA virus (HSV-1), and RNA virus (HPeV). All samples were prepared in a CSF matrix. Serial dilutions were prepared for both single-spike samples and multiple spiked samples containing the corresponding analyte for comparison.

All sample dilutions were prepared using artificial CSF. Testing was conducted over 3 days by different operators using at least 5 different QIAstat-Dx ME Panel lots on 3 or more QIAstat-Dx Analyzer 1.0 instruments.

In addition to meeting the acceptance criteria for qualitative performance, there should be no significant differences in assay metrics (i.e., Ct values) between samples prepared in the individual and combined samples.

**Table 16: Multiplexed Limit of Detection Expected Results**

Concentration*	Minimum Number of Replicates per Strain	Expected Results (% positive)
5x	10	100
1x	30	≥95
0.1x	10	10-90
Negative	10	0

\*LoD as determined in individual LoD study.

No significant differences that negatively impacted assay performance were observed.

8. Assay Cut-Off:  
Not applicable.

## **B Comparison Studies:**

1. Method Comparison with Predicate Device:  
N/A

2. Matrix Comparison:

A matrix equivalency study was conducted to compare the performance of analytical samples prepared in negative clinical cerebrospinal fluid (cCSF) matrix to sample prepared in artificial CSF matrix (aCSF). A total of 26 pathogen strains (at least two different strains per panel target) were tested in combined samples spiked in true-negative cCSF. Clinical CSF was sourced from commercial suppliers and was tested prior to its use in mix manufacture for true-negativity with either the QIAstat-Dx ME Panel or an alternative method.

Samples were prepared by spiking pathogens in multiplex sample mixes containing up to 5 organisms per sample in cCSF at 5x LoD, 1x LoD and 0.1x LoD concentrations following the Limit of Detection in Combined Samples study (See Section 7.ii.). The concentration of the pathogen strain that achieved a result around the detection limit (1x LoD) was assessed by testing a minimum of 30 replicates; 5x LoD and 0.1x LoD concentrations were assessed by testing a minimum of 10 replicates. Some pathogens required an additional round of testing to establish the dilution to achieve LoD, in addition to the initial testing described.

Testing for each concentration was executed during at least 3 different days by different operators using four different lots of QIAstat-Dx ME Panel cartridges executed on 3 or

more QIAstat-Dx Analyzers. Negative samples consisted of unspiked cCSF, and a minimum of 10 replicates were tested.

To demonstrate equivalence, in addition to meeting the acceptance criteria for qualitative performance, there should be no significant differences in assay metrics (i.e., Ct values). The difference in mean target Ct values between the two sample matrix types, evaluated with an ANOVA fitted test, will not be statistically significant when p-value >0.05. If statistically significant, then the mean difference in Ct value of the combined sample in cCSF compared to the combined samples in aCSF should be within  $\pm 2 \times$  SD of the combined sample in cCSF to be deemed equivalent.

Of the 26 pathogen strains evaluated, the LoD concentration was equivalent between combined (multi-spiked) samples in aCSF and cCSF in 17 strains. Nine pathogen strains (*Streptococcus pneumoniae* (ZeptoMetrix 0801439), *Streptococcus pyogenes* (ZeptoMetrix 0804351), Enterovirus B (ZeptoMetrix 0810019CF), Enterovirus C (ATCC VR-583), Enterovirus D (ATCC VR-1823)) were not equivalent and had a new LoD concentration determined in cCSF.

## C Clinical Studies:

### 1. Prospective Clinical Sensitivity:

The clinical performance of the Qiagen QIAstat-Dx ME Panel to detect and identify target bacteria, fungi, and viruses from cerebrospinal fluid samples was assessed in a multi-site study using residual de-identified CSF samples. Testing was conducted across 13 geographically diverse sites (10 U.S. and 3 European) using residual cerebrospinal fluid specimens from patients with signs and symptoms of meningitis and/or encephalitis. Performance for the QIAstat-Dx ME Panel was compared to the predicate device, BioFire FilmArray ME Panel except for the target not detected by the predicate device, *Streptococcus pyogenes*, which were compared to PCR followed by bidirectional sequencing.

Testing of prospectively collected residual specimens occurred between March 2022 – March 2023. A total of 1737 specimens were enrolled with 205 withdrawn, most commonly for ineligibility (not meeting inclusion/exclusion criteria). To supplement results of the prospective clinical study, frozen archived positive specimens were blinded and mixed with prospective specimens at clinical sites. Contrived samples were assigned a unique blinded specimen ID prior to being randomized and shipped to investigator sites along with negative contrived samples.

Prior to testing at the clinical sites, prospective cerebrospinal fluid specimens were stored up to 24 hours at room temperature (15-25°C), up to 7 days at 2-8°C, or frozen ( $\leq -70^\circ\text{C} \pm 20^\circ\text{C}$ ) prior to testing. After comparator testing at the study site, all specimens were frozen ( $\leq -70^\circ\text{C} \pm 20^\circ\text{C}$ ) and stored before being shipped to central testing sites for additional comparator testing or discordance testing, if required. Discordant results were analyzed using Standard of Care (SoC) culture when sufficient sample volume remained.

The study inclusion criteria included the following:

- Sites were advised to make best effort to provide unique specimens (only one sample per patient).
- Specimens must have met the laboratory testing criteria for individuals with signs and/or symptoms of meningitis and/or encephalitis. Specimens consisting of only CSF obtained via lumbar puncture were collected.
- Specimens were residual and should not have undergone more than three (3) freeze/thaw cycles.
- Minimum 500 µL of residual volume.
- Fresh specimens should be stored at 2-8°C for 7 days. Ship within to testing sites within 48 hours of collection (for testing at sponsor site / testing site within total 72 hours post collection). Frozen specimens should be stored at -70°C (±20°C) up to 2 months.

The study exclusion criteria included the following:

- Specimens not fitting inclusion criteria outlined above
- Cerebrospinal fluid obtained from an external ventricular drain or shunt source
- Specimen has been centrifuged
- Obvious physical damage of banked residual specimen
- Repeat specimens from the same subject

**Table 17: Demographic Summary for Evaluable Prospective Samples**

Sample Type	Variable	Subgroup	N	(%)
Prospective Fresh	Age Group	<1 year	136	14.0%
		1-17 years old	87	9.0%
		18-44 years old	284	29.2%
		45-64 years old	266	27.4%
		65-84 years old	187	19.2%
		≥85 years old	11	1.1%
		Unknown	1	0.1%
	Gender	Female	498	51.2%
		Male	474	48.8%
Prospective Frozen	Age Group	<1 year	27	4.9%
		1-17 years old	41	7.4%
		18-44 years old	133	24.1%
		45-64 years old	174	31.5%
		65-84 years old	156	28.3%
		≥85 years old	20	3.6%
		Unknown	1	0.2%
	Gender	Female	271	49.1%
		Male	280	50.7%
		Not Available	1	0.2%
Combined	Age Group	<1 year	163	10.7%
		1-17 years old	128	8.4%

		18-44 years old	417	27.4%
		45-64 years old	440	28.9%
		65-84 years old	343	22.5%
		≥85 years old	31	2.0%
		Unknown	2	0.1%
	Gender	Female	769	50.5%
		Male	754	49.5%
		Not Available	1	0.1%

**Table 18: Prospective Clinical Performance Summary**

Target		Positive Percent Agreement			Negative Percent Agreement		
		TP / (TP+FN)	(%)	95% CI	TN / (TN+FP)	(%)	95% CI
Bacteria							
<i>Escherichia Coli</i> K1	Fresh	2/3 <sup>a</sup>	66.7%	20.8%-93.9%	969/969	100.0%	99.6%-100.0%
	Frozen	0/1 <sup>a</sup>	0.0%	0.0%-79.3%	551/551	100.0%	99.3%-100.0%
	Overall	2/4	50.0%	15.0%-85.0%	1520/1520	100.0%	99.7%-100.0%
<i>Haemophilus influenzae</i>	Fresh	0/1 <sup>b</sup>	0.0%	0.0%-79.3%	970/971 <sup>b</sup>	99.9%	99.4%-100.0%
	Frozen	4/4	100.0%	51.0%-100.0%	546/548 <sup>b</sup>	99.6%	98.7%-99.9%
	Overall	4/5	80.0%	37.6%-96.4%	1516/1519	99.8%	99.4%-99.9%
<i>Listeria monocytogenes</i>	Fresh	1/1	100.0%	20.7%-100.0%	971/971	100.0%	99.6%-100.0%
	Frozen	3/4 <sup>c</sup>	75.0%	30.1%-95.4%	548/548	100.0%	99.3%-100.0%
	Overall	4/5	80.0%	37.6%-96.4%	1519/1519	100.0%	99.7%-100.0%
<i>Neisseria meningitidis</i> (encapsulated)	Fresh	1/1	100.0%	20.7%-100.0%	971/971	100.0%	99.6%-100.0%
	Frozen	0/0	N/A	N/A	551/552 <sup>d</sup>	99.8%	99.0%-100.0%
	Overall	1/1	100.0%	20.7%-100.0%	1522/1523	99.9%	99.6%-100.0%
<i>Streptococcus agalactiae</i>	Fresh	2/2	100.0%	34.2%-100.0%	970/970	100.0%	99.6%-100.0%
	Frozen	1/1	100.0%	20.7%-100.0%	551/551	100.0%	99.3%-100.0%
	Overall	3/3	100.0%	43.9%-100.0%	1521/1521	100.0%	99.7%-100.0%
<i>Streptococcus pneumoniae</i>	Fresh	1/1	100.0%	20.7%-100.0%	845/848 <sup>e</sup>	99.6%	99.0%-99.9%

	Frozen	7/7	100.0%	64.6%-100.0%	515/517 <sup>c</sup>	99.6%	98.6%-99.9%
	Overall	8/8	100.0%	67.6%-100.0%	1360/1365	99.6%	99.1%-99.8%
<i>Streptococcus pyogenes</i>	Fresh	0/0	N/A	N/A	778/778	100.0%	99.5%-100.0%
	Frozen	0/0	N/A	N/A	513/513	100.0%	99.3%-100.0%
	Overall	0/0	N/A	N/A	1291/1291	100.0%	99.7%-100.0%
<b>Virus</b>							
Enterovirus (EV)	Fresh	18/20 <sup>f</sup>	90.0%	69.9%-97.2%	951/952 <sup>f</sup>	99.9%	99.4%-100.0%
	Frozen	4/4	100.0%	51.0%-100.0%	548/548	100.0%	99.3%-100.0%
	Overall	22/24	91.7%	74.2%-97.7%	1499/1500	99.9%	99.6%-100.0%
<b>Fungi / Yeast</b>							
<i>Cryptococcus gattii</i> / <i>Cryptococcus neoformans</i> (not differentiated)	Fresh	2/5 <sup>g</sup>	40.0%	11.8%-76.9%	965/967 <sup>g</sup>	99.8%	99.2%-99.9%
	Frozen	2/2	100.0%	34.2%-100.0%	550/550	100.0%	99.3%-100.0%
	Overall	4/7	57.1%	25.0%-84.2%	1515/1517	99.9%	99.5%-100.0%

<sup>a</sup> For the prospective fresh *Escherichia coli* K1 discordant sample, no organisms were detected with PCR/BDS. For the frozen *Escherichia coli* K1 discordant sample no organisms were detected with bacterial culture.

<sup>b</sup> For the prospective fresh *Haemophilus influenzae* discordant sample, no organisms were detected by the SoC bacterial culture and testing with PCR/BDS. Of the three (3) false positive *Haemophilus influenzae* samples, no organisms were detected in one fresh and one frozen by SoC culture and PCR/BDS was also negative. No additional testing results associated with the final frozen false positive sample were available.

<sup>c</sup> For the frozen *Listeria monocytogenes* discordant sample the negative result was confirmed positive with SoC culture and LDT result was positive.

<sup>d</sup> For the frozen *Neisseria meningitidis* (encapsulated) sample, no organisms were detected by SoC culture and LDT testing with PCR/BDS also returned a negative result for this sample.

<sup>e</sup> Of the five (5) false positive *Streptococcus pneumoniae* samples, no organisms were detected in four (3 fresh and 1 frozen) prospective samples with SoC culture. One prospective frozen sample had no SoC result available. However, an FDA cleared method conducted as part of the study also produced a negative result.

<sup>f</sup> For the prospective fresh Enterovirus discordant samples, no organisms were detected in one sample by two independent SoC LDT assays. The negative result for the second sample returned a negative result with PCR/BDS. For the prospective fresh false positive Enterovirus sample, a negative result was returned when tested with PCR/BDS.

<sup>g</sup> Of the three false negative *Cryptococcus gattii* / *Cryptococcus neoformans* (not differentiated) results, no organisms were detected in two fresh samples with fungal culture and PCR/BDS. The remaining false negative fresh sample was confirmed negative for *Cryptococcus gattii* / *Cryptococcus neoformans* (not differentiated) with PCR/BDS. Of the two false positive results, no organisms were detected for one fresh sample with PCR/BDS. No organisms were detected in the second fresh sample with SoC fungal culture.

## 2. Retrospective Samples

Several targets detected by the QIAstat-Dx ME Panel were not encountered during the prospective clinical study in sufficient numbers to demonstrate assay performance. Therefore, the prospective clinical study was supplemented with additional testing using retrospective positive samples characterized previously by a standard of care molecular

assay. A summary of the demographic information for retrospective samples is presented in Table 19.

**Table 19: Retrospective Samples Demographic Data**

Sample Type	Variable	Subgroup	N	(%)
Archived	Age Group	<1 year	11	26.8%
		1-17 years old	9	22.0%
		18-44 years old	12	29.3%
		45-64 years old	5	12.2%
		65-84 years old	4	9.8%
	Gender	Female	19	46.3%
		Male	22	53.7%

The performance of the QIAstat-Dx ME Panel was determined by comparing to an FDA-cleared molecular assay for all retrospective samples and summarized in Table 20. All retrospective samples generated a valid result in the first attempt.

**Table 20: Retrospective Clinical Performance Summary**

Target	Positive Percent Agreement			Negative Percent Agreement		
	TP / (TP+FN)	(%)	95% CI	TN / (TN+FP)	(%)	95% CI
<b>Bacteria</b>						
<i>Escherichia Coli</i> K1	2/2	100.0%	34.2%-100.0%	39/39	100.0%	91.0%-100.0%
<i>Haemophilus influenzae</i>	6/6	100.0%	61.0%-100.0%	35/35	100.0%	90.1%-100.0%
<i>Listeria monocytogenes</i>	0/0	N/A	N/A	41/41	100.0%	91.4%-100.0%
<i>Neisseria meningitidis</i> (encapsulated)	3/3	100.0%	43.9%-100.0%	38/38	100.0%	90.8%-100.0%
<i>Streptococcus agalactiae</i>	9/9	100.0%	70.1%-100.0%	32/32	100.0%	89.36%-100.0%
<i>Streptococcus pneumoniae</i>	4/4	100.0%	51.0%-100.0%	18/18	100.0%	82.4%-100.0%
<i>Streptococcus pyogenes</i>	0/0	N/A	N/A	23/23	100.0%	85.7%-100.0%
<b>Virus</b>						
Enterovirus (EV)	9/9	100.0%	70.1%-100.0%	32/32	100.0%	89.3%-100.0%
<b>Fungi / Yeast</b>						
<i>Cryptococcus gattii</i> / <i>Cryptococcus neoformans</i> (not differentiated)	8/8	100.0%	67.6%-100.0%	33/33	100.0%	89.6%-100.0%

### 3. Contrived Samples

Contrived specimens were prepared for each target. *Streptococcus pyogenes* was not observed in the prospective study and is supported solely with contrived samples. Contrived samples were prepared by spiking five quantified strains representative of the genetic diversity of each pathogen (Table 21) at a concentration of 2x and 5x LoD in negative cerebrospinal fluid. Contrived specimens were mixed with prospective specimens, stored at -70°C (±20°C), and tested in batches as received by the clinical testing sites. It was not possible to test contrived samples alongside prospective samples for 676 samples. An equal number of negative samples were randomly mixed with the prepared samples to maintain blinded conditions.

**Table 21: Contrived Target Strains**

Pathogen	Strain	Supplier	Catalogue ID
<i>Cryptococcus gattii</i> / <i>Cryptococcus neoformans</i>	WM629 [CBS 10079]	ATCC	MYA-4567
<i>Cryptococcus gattii</i> / <i>Cryptococcus neoformans</i>	A1M R272	ATCC	MYA-4094
<i>Cryptococcus gattii</i> / <i>Cryptococcus neoformans</i>	C. neoformans H99	ATCC	208821
<i>Cryptococcus gattii</i> / <i>Cryptococcus neoformans</i>	Alg254	BEI Resources	NR-50198
<i>Cryptococcus gattii</i> / <i>Cryptococcus neoformans</i>	A6MR38 [CBS 11545]	ATCC	MYA-4877
Enterovirus	Coxsackievirus A16	Zeptomatrix	0810107CF
Enterovirus	Coxsackievirus B5	Zeptomatrix	0810019CF
Enterovirus	G-12 NIAID V-020-002-062	ATCC	VR-1023
Enterovirus	US/MO/14-18947	ATCC	VR-1823
Enterovirus	EV 70, species D, strain J670/71	ATCC	VR-836
<i>Escherichia coli</i> K1	Strain C5 [Bort]; O18ac:K1:H7	ATCC	700973
<i>Escherichia coli</i> K1	O-16, F1119-41	BEI Resources	NR-17674
<i>Escherichia coli</i>	K1 O-2, U9-41	BEI Resources	NR-17666
<i>Escherichia coli</i> K1	NCDC F 11119-41	ATCC	23511
<i>Escherichia coli</i> K1	NCTC 9001. Serovar O1:K1:H7	ATCC	11775
<i>Haemophilus influenzae</i>	type b (cap)	ATCC	10211
<i>Haemophilus influenzae</i>	Type a [strain AMC 36-A-3]	ATCC	9006
<i>Haemophilus influenzae</i>	Type f [strain GA-1264]	ATCC	700223
<i>Haemophilus influenzae</i>	AMC 36-A-7	ATCC	8142
<i>Haemophilus influenzae</i>	Type c [strain C 9007]	ATCC	49699
<i>Listeria monocytogenes</i>	Strain Li2	ATCC	19115
<i>Listeria monocytogenes</i>	Serotype 1/2b	Zeptomatrix	801534
<i>Listeria monocytogenes</i>	Strain Li 20	ATCC	19111
<i>Listeria monocytogenes</i>	2011L-2676	ATCC	BAA-2659
<i>Listeria monocytogenes</i>	Serotype 4b	Zeptomatrix	804339
<i>Neisseria meningitidis</i>	M2092	ATCC	13090
<i>Neisseria meningitidis</i>	M-112	ATCC	35561
<i>Neisseria meningitidis</i>	Serotype B. M997 [S-3250-L]	ATCC	13092

<i>Neisseria meningitidis</i>	Serotype D. M158 [37A]	ATCC	13113
<i>Neisseria meningitidis</i>	M-1574 [199/W135]	ATCC	43744
<i>Streptococcus agalactiae</i>	G19 group B	ATCC	13813
<i>Streptococcus agalactiae</i>	Z019	ZeptoMetrix	801545
<i>Streptococcus agalactiae</i>	type III-ST283	ATCC	31475
<i>Streptococcus agalactiae</i>	Z023	ZeptoMetrix	801556
<i>Streptococcus agalactiae</i>	2603 V/R. Serotype V	ATCC	BAA-611
<i>Streptococcus pneumoniae</i>	19F	ZeptoMetrix	801439
<i>Streptococcus pneumoniae</i>	Serotype 1. NCTC 7465	ATCC	33400
<i>Streptococcus pneumoniae</i>	Serotype 5. SPN1439-106 [Colombia 5-19]	ATCC	BAA-341
<i>Streptococcus pneumoniae</i>	Serotype 11A. Type 43	ATCC	10343
<i>Streptococcus pneumoniae</i>	Serotype 14. VH14	ATCC	700672
<i>Streptococcus pyogenes</i>	Z472; Serotype M1	Zeptomatrix	804351
<i>Streptococcus pyogenes</i>	Bruno [CIP 104226]	ATCC	19615
<i>Streptococcus pyogenes</i>	Z018; Serotype M58	ZeptoMetrix	801512
<i>Streptococcus pyogenes</i>	Lancefield's group A / C203 S	ATCC	14289
<i>Streptococcus pyogenes</i>	Serotype M1. MGAS 5005	ATCC	BAA-947

**Table 22: Contrived Samples Performance Summary**

Target		Positive Percent Agreement		
		TP / (TP+FN)	(%)	95% CI
<b>Bacteria</b>				
<i>Escherichia Coli</i> K1	2xLoD	48/48	100.0%	92.6%-100.0%
	5xLoD	37/37	100.0%	90.6%-100.0%
	Contrived	85/85	100.0%	95.7%-100.0%
<i>Haemophilus influenzae</i>	2xLoD	57/57	100.0%	93.7%-100.0%
	5xLoD	36/36	100.0%	90.4%-100.0%
	Contrived	93/93	100.0%	96.0%-100.0%
<i>Listeria monocytogenes</i>	2xLoD	47/49	95.9%	86.3%-100.0%
	5xLoD	38/38	100.0%	90.8%-100.0%
	Contrived	85/87	97.7%	92.0%-100.0%
<i>Neisseria meningitidis</i> (encapsulated)	2xLoD	46/48	95.8%	86.0%-100.0%
	5xLoD	39/40	97.5%	87.1%-100.0%
	Contrived	85/88	96.6%	90.5%-100.0%
<i>Streptococcus agalactiae</i>	2xLoD	49/49	100.0%	92.7%-100.0%
	5xLoD	39/39	100.0%	91.0%-100.0%
	Contrived	88/88	100.0%	95.8%-100.0%
<i>Streptococcus pneumoniae</i>	2xLoD	55/57	96.5%	88.1%-100.0%
	5xLoD	39/39	100.0%	91.0%-100.0%
	Contrived	94/96	97.9%	92.7%-100.0%
<i>Streptococcus pyogenes</i>	2xLoD	47/49	95.9%	86.3%-100.0%
	5xLoD	40/40	100.0%	91.2%-100.0%
	Contrived	87/89	97.8%	92.2%-100.0%

Virus				
Enterovirus (EV)	2xLoD	48/49	98.0%	89.3%-100.0%
	5xLoD	39/39	100.0%	91.0%-100.0%
	Contrived	87/88	98.9%	93.8%-100.0%
Fungi / Yeast				
<i>Cryptococcus gattii</i> / <i>Cryptococcus neoformans</i> (not differentiated)	2xLoD	41/41	100.0%	91.4%-100.0%
	5xLoD	38/38	100.0%	90.8%-100.0%
	Contrived	79/79	100.0%	95.4%-100.0%

**D Clinical Cut-Off:**

Not applicable.

**E Expected Values/Reference Range:**

The QIAstat-Dx ME Panel assay prospective clinical study was conducted to evaluate clinical performance of the device using cerebrospinal fluid samples. The number and percentage of positive results as determined by the QIAstat-Dx ME Panel, stratified by age group are presented in Table 23. Overall, 1527 specimens were included in the prevalence assessment and the QIAstat-Dx ME Panel detected at least one organism in a total of 65 prospective specimens analyzed in the study (4.3% positivity rate).

**Table 23: Expected Values by Age Group (Prospective Samples)**

Pathogen	Overall	Age (years)						
		<1	1-17	18-44	45-64	65-84	>85	Unknown
Bacteria								
<i>Escherichia coli</i> K1	0.1% (2)	100.0% (2/2)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)
<i>Haemophilus influenzae</i>	0.5% (7)	0.0% (0)	0.0% (0)	0.2% (1)	1.1% (5)	0.3% (1)	0.0% (0)	0.0% (0)
<i>Listeria monocytogenes</i>	0.3% (4)	0.0% (0)	0.0% (0)	0.0% (0)	0.5% (2)	0.3% (1)	3.2% (1)	0.0% (0)
<i>Neisseria meningitidis</i> (encapsulated)	0.1% (2)	0.0% (0)	0.0% (0)	0.2% (1)	0.2% (1)	0.0% (0)	0.0% (0)	0.0% (0)
<i>Streptococcus agalactiae</i>	0.2% (3)	0.6% (1)	0.0% (0)	0.0% (0)	0.5% (2)	0.0% (0)	0.0% (0)	0.0% (0)
<i>Streptococcus pneumoniae</i>	1.1% (17)	1.8% (3)	0.0% (0)	0.7% (3)	1.6% (7)	1.2% (4)	0.0% (0)	0.0% (0)
<i>Streptococcus pyogenes</i>	0.1% (1)	0.0% (0)	0.8% (1)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)
Virus								
Enterovirus	1.5% (23)	7.4% (12)	2.3% (3)	1.2% (5)	0.2% (1)	0.3% (1)	0.0% (0)	50.0% (1)
Yeast								

<i>Cryptococcus gattii</i> / <i>Cryptococcus</i> <i>neoformans</i>	0.4% (6)	0.0% (0)	0.0% (0)	0.2% (1)	1.1% (5)	0.0% (0)	0.0% (0)	0.0% (0)
<b>Overall Panel Result</b>								
Negative	95.7% (1462)	89.0% (145)	96.9% (124)	97.4% (407)	94.8% (419)	98.0% (336)	96.8% (30)	50.0% (1)
Positive	4.3% (65)	11.0% (18)	3.1% (4)	2.6% (11)	5.2% (23)	2.0% (7)	3.2% (1)	50.0% (1)

**VIII Proposed Labeling:**

The labeling supports the finding of substantial equivalence for this device.

**IX Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.