October 29, 2024

QIAGEN GmbH
% Sonia Pablo Pablo
Senior Manager, Regulatory Affairs
STAT-Dx Life, S.L. (A QIAGEN Company)
Carrer Baldiri Reixac, 4
Barcelona, 08028
Spain

Re: K242256
Trade/Device Name: QIAstat-Dx Meningitis/Encephalitis (ME) Panel
Regulation Number: 21 CFR 866.3970
Regulation Name: Device To Detect And Identify Microbial Pathogen Nucleic Acids In Cerebrospinal Fluid
Regulatory Class: Class II
Product Code: PLO
Dated: July 29, 2024
Received: July 31, 2024

Dear Sonia Pablo Pablo:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device"

(https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Bryan M. Grabias -S

Digitally signed by Bryan M. Grabias -S
Date: 2024.10.29 15:52:56 -04'00'

Bryan Grabias
Acting Branch Chief
Bacterial Respiratory and Medical Countermeasures Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K242256

Device Name
QIAstat-Dx Meningitis/Encephalitis (ME) Panel

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

510(k) Summary

General Information

Submitted by: QIAGEN GmbH
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Contact Person: Sonia Pablo
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Spain
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Date Prepared: October 28, 2024

Device Name: QIAstat-Dx® Meningitis/Encephalitis (ME) Panel

Classification: 21 CFR 866.3970 - Device to detect and identify microbial pathogen nucleic acids in cerebrospinal fluid.

Product Code: PLO

Predicate Device:

Manufacturer	Product Name	De Novo/510(k) No.
BioFire Diagnostics, LLC	FilmArray Meningitis/Encephalitis (ME) Panel	K160462

Device Description

The QIAstat-Dx® Meningitis/Encephalitis (ME) Panel is part of the QIAstat-Dx Meningitis/Encephalitis system and works with the QIAstat-Dx Analyzer 1.0.

The QIAstat-Dx ME Panel is intended to be used with cerebrospinal fluid (CSF) specimens.

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 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 detected analytes are displayed in red. For other analytes tested, they are displayed in green if not detected or in gray if not applicable or invalid. The analyzer will report if an error occurs during processing, in which case the test will fail and no results will be provided (screen will show "FAIL").

QIAstat-Dx consists of single-test cartridges with pre-packaged reagents including both wet and dry chemistry necessary to perform the sample preparation, nucleic acid amplification and detection to be used in conjunction with the QIAstat-Dx Analyzer 1.0. All sample preparation and assay steps are performed within the cartridge, so the user does not need to manipulate any reagent during the test. This eliminates exposure of the user or the Analyzer to chemicals contained in the cartridge during the test and up to the disposal of used cartridges.

Within the cartridge, multiple steps are automatically performed in sequence by using pneumatic pressure and a multiport valve to transfer the sample and fluids via the Transfer Chamber (TC) to their intended destinations. Following the introduction of the sample from a disposable transfer pipette, the following assay steps occur automatically and sequentially:

- Resuspension of air-dried internal control and Proteinase K (ProtK) enzyme using provided buffer and mixing with the liquid sample (IC Cavity and ProtK Cavity);
- Cell lysis using mechanical (rotation) and chemical (chaotropic and isotonic) means (lysis chamber);
- Membrane-based nucleic acid purification from Lysate by:
    - Mixing lysate with binding buffer and capturing on the membrane (purification chamber);
    - First washing of membrane to remove bound proteins (purification chamber and waste chamber);
    - Second washing of membrane to leave only bound nucleic acids (purification chamber and waste chamber);
    - Rinsing of Transfer Chamber (TC) using the rinsing buffer before introduction of the eluate (Transfer Chamber);

  - Drying of membrane with bound nucleic acids with an air flow generated by a high flow vacuum pump (purification chamber); and
  - Elution of nucleic acids with elution buffer (purification chamber and TC);
- Mixing of the purified nucleic acid (eluate) with lyophilized "Master Mix" reagents (Dry chemistry container (DCC) and TC);
- Sequential transfer of defined aliquots of mixed eluate/Master Mix from the Transfer Chamber to each of eight Reaction Chambers containing the specified, air-dried primers and probes;
- Within each Reaction Chamber, real-time, multiplex PCR ("rtPCR") testing is performed. Increase in fluorescence (indicative of detection of each target analyte) is detected directly within each Reaction Chamber; and
- The detected signal per fluorescent marker per Reaction Chamber is then used by the system software to generate the assay result.

Intended Use

The QIAstat-Dx® Meningitis/Encephalitis (ME) Panel is a qualitative multiplexed nucleic acid real-time PCRbased 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 (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 instructions for use.

The QIAstat-Dx ME Panel is not intended for testing of specimens collected from indwelling CNS 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.

Comparison of the QIAstat-Dx ME Panel and the Predicate Device

Similarities and differences between the QIAstat-Dx ME Panel and the predicate device are shown in Table 1.

Table 1: Comparison of the QIAstat-Dx ME Panel with the predicate device

Characteristic	Device	Predicate
Name	QIAstat-Dx ME Panel	FilmArray Meningitis/Encephalitis (ME) Panel
510(k) No.	K242256	K160462
Regulation	21 CFR 866.3970	21 CFR 866.3970
Product Code	PLO	PLO
Device Class	Class II	Class II
Similarities		
Intended 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: Bacteria: • *Escherichia col*i K1 • *Haemophilus influenzae* • *Listeria monocytogenes*	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. The following organisms are identified using the FilmArray ME Panel: Bacteria: • *Escherichia coli* K1 • *Haemophilus influenzae* • *Listeria monocytogenes*

Characteristic	Device	Predicate
	• *Neisseria meningitidis* (encapsulated) • *Streptococcus agalactiae* • *Streptococcus pneumoniae* • *Streptococcus pyogenes* Virus: • Enterovirus Yeast: • *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 (CNS) infection. Not all agents of CNS infection are detected by this test and sensitivity in clinical use may	• *Neisseria meningitidis* (encapsulated) • *Streptococcus agalactiae* • *Streptococcus pneumoniae* Viruses: • Enterovirus • Cytomegalovirus • 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

Characteristic	Device	Predicate
	differ from that described in the instructions for use. The QIAstat-Dx ME Panel is not intended for testing of specimens collected from indwelling CNS 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.	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 is intended to be used in conjunction with standard of care culture for organism recovery, serotyping, and antimicrobial susceptibility testing.
Specimen Type	Cerebrospinal Fluid	Cerebrospinal Fluid
Analyte Detected	RNA/DNA	RNA/DNA
Organisms Detected	See above	See above
Amplification and Detection Technology	PCR	PCR
Differences		
Assay Controls	One internal control in each cartridge to control for sample processing that is subjected to all nucleic acid extraction and amplification steps similar to patient samples. Labeling will recommend use of negative and positive external controls regularly. Use transport media as the external negative control, and previously characterized positive samples or negative sample spiked with well characterized target organisms as external positive controls.	Two controls are included in each reagent pouch to control for sample processing and both stages of PCR and melt analysis. Labeling recommends the use of external positive and negative controls regularly. Enteric transport media can be used as an external negative control, and previously characterized positive samples or negative samples spiked with well characterized organisms as external positive controls.

Characteristic	Device	Predicate
Assay Targets	The QIAstat-Dx ME Panel detects: Bacteria: • *Streptococcus pyogenes*	The FilmArray ME Panel detects: Viruses: • Cytomegalovirus • Herpes simplex virus 1 • Herpes simplex virus 2 • Human herpesvirus 6 • Human parechovirus • Varicella zoster virus
Nucleic Acid Extraction	Extraction of nucleic acids using silica membrane	Extraction of nucleic acids using standard magnetic beads
Technology	QIAstat-Dx detection of amplified targets uses an increase in fluorescence to generate the assay results.	The FilmArray ME Panel uses melting curve analysis to confirm the identity of amplified targets to generate assay results.
Operational	The sample is loaded straight into the cartridge.	The FilmArray ME Panel must rehydrate and prime the cartridge before use.
Amplification and Detection Instrument System	QIAstat-Dx Analyzer 1.0	FilmArray Instruments

Performance Characteristics - Non-clinical Studies

The studies presented have been performed to demonstrate the non-clinical performance of the QIAstat-Dx ME Panel.

Limit of Detection

The Limit of Detection (LoD) is defined as the lowest concentration at which ≥95% of samples tested generate a positive call.

The LoD for each QIAstat-Dx ME Panel target was assessed, using 26 pathogen strains, by analyzing dilutions of analytical samples prepared from stocks obtained from commercial suppliers. The LoD was determined using selected strains representing individual pathogens that are possible to detect with the QIAstat- Dx ME Panel. All sample dilutions were prepared using artificial CSF. To confirm the established LoD concentration, the required detection rate of all replicates was ≥95%. Additional testing of samples prepared using negative clinical CSF was conducted to assess equivalency.

Individual LoD concentrations for each QIAstat-Dx ME Panel target are shown in Table 2.

Table 2: LoD concentrations for the strains tested with QIAstat-Dx ME Panel

Pathogen	Strain	Source / Catalog ID	LoD Concentration*	Detection Rate
Escherichia coli K1	Strain C5 [Bort]; O18ac:K1:H7	ATCC 700973	3.48E+02 CFU/mL	30/30
Escherichia coli K1	NCTC 9001. Serovar O1:K1:H7	ATCC 11775	7.86E+02 CFU/mL	30/30
Haemophilus influenzae	type b (cap)	ATCC 10211	3.16E+02 CFU/mL	32/32
Haemophilus influenzae	Type e [strain AMC 36-A-7]	ATCC 8142	2.54E+03 CFU/mL	30/30
Listeria monocytogenes	Type 1/2b	ZeptoMetrix 0801534	1.86E+03 CFU/mL	21/21
Listeria monocytogenes	Type 4b. Strain Li 2	ATCC 19115	6.64E+03 CFU/mL	30/30
Neisseria meningitidis (encapsulated)	Serotype B. M2092	ATCC 13090	8.28E-02 CFU/mL	31/32
Neisseria meningitidis (encapsulated)	Serotype Y. M-112 [BO-6]	ATCC 35561	1.33E+01 CFU/mL	30/30
Streptococcus agalactiae	Z019	ZeptoMetrix 0801545	1.75E+03 CFU/mL	30/30
Streptococcus agalactiae	G19 group B	ATCC 13813	3.38E+03 CFU/mL	31/31
Streptococcus pneumoniae	19F	ZeptoMetrix 0801439	7.14E+02 CFU/mL	29/30
Streptococcus pneumoniae	Serotype 1. NCTC 7465	ATCC 33400	6.22E-01 CFU/mL	29/29
Streptococcus pyogenes	Z472; Serotype M1	ZeptoMetrix 0804351	1.80E+03 CFU/mL	30/30
Streptococcus pyogenes	Bruno [CIP 104226]	ATCC 19615	9.10E+01CFU/mL	30/30
Enterovirus A	Coxsackievirus A16	ZeptoMetrix 0810107CF	3.79E+00 TCID50/mL	31/31
Enterovirus A	A6, species A. Strain Gdula	ATCC VR-1801	1.60E+02 TCID50/mL	30/30
Enterovirus B	Coxsackievirus B5	ZeptoMetrix 0810019CF	8.91E+01 TCID50/mL	30/30
Enterovirus B	Coxsackievirus A9, species B	ZeptoMetrix 0810017CF	4.36E+01 TCID50/mL	28/29
Enterovirus C	Coxsackievirus A17, species C. Strain G-12	ATCC VR-1023	1.58E+01 TCID50/mL	30/30
Enterovirus C	Coxsackievirus A24. Starin DN-19	ATCC VR-583	4.99E+00 TCID50/mL	30/30

Pathogen	Strain	Source / Catalog ID	LoD Concentration*	Detection Rate
Enterovirus D	EV 70, species D, strain J670/71	ATCC VR-836	4.99E+01 TCID50/mL	30/31
Enterovirus D	Enterovirus D68. Strain US/MO/14-18947	ATCC VR-1823	5.06E+02 TCID50/mL	30/30
Cryptococcus neoformans	Serotype D strain WM629, type VNIV	ATCC MYA-4567	2.21E+03 CFU/mL	31/31
Cryptococcus neoformans	*C. neoformans* H99	ATCC 208821	1.64E+02 CFU/mL	31/31
Cryptococcus gattii	Serotype B strain R272, type VGIIb	ATCC MYA-4094	1.32E+04 CFU/mL	30/30
Cryptococcus gattii	A6MR38 [CBS 11545]	ATCC MYA-4877	2.60E+03 CFU/mL	29/29

*Highest LoD concentration from LoD/Matrix Equivalency studies is reported.

Analytical Reactivity (Inclusivity)

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 Limit of Detection.

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 performed in two steps (*in vitro* and *in silico*).

Based on *in vitro* (wet) and *in silico* analysis, the QIAstat-Dx ME Panel has demonstrated a broad coverage for all targets detected by the panel.

In vitro analysis
A total of 130 strains covering 10 different analytical strains for each targeted organism or relevant species were tested spiked in combined samples. Strains tested for inclusivity are shown in Table 3.

125 out of 130 pathogen strains were successfully detected by the QIAstat-Dx ME Panel when tested *in vitro*. Five strains were not detected by the assay (detailed in Table 4).

Table 3: Strains tested for Inclusivity

Pathogen	Source (Catalog ID)	Strain/Serotype	Lowest Concentration Tested
Escherichia coli K1	ATCC (700973) [a]	Strain C5 [Bort]; O18ac:K1:H7	1x LoD
	ATCC (11775) [a]	NCTC 9001. Serovar O1:K1:H7	1x LoD
	NCTC (9007)	Strain Bi 7509/41; O7:K1:H-	0.03x LoD

Pathogen	Source (Catalog ID)	Strain/Serotype	Lowest Concentration Tested
	ATCC (23509)	NCDC Bi 7509-41 Serotype O7 :K1(L) :NM	1:100 from stock
	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
Haemophilus influenzae	ATCC (10211) [a]	type b (cap)	1x LoD
	ATCC (8142) [a]	Type e [strain AMC 36-A-7]	1x LoD
	ATCC (51907)	Non-typeable [strain Rd [KW20]	0.014x LoD
	ATCC (11116)	Non-typeable [strain 180-a]	3x LoD
	ATCC (9006)	Type a [strain AMC 36-A-3]	3x LoD
	ATCC (31512)	Type b [strain Rab]	0.016x LoD
	ATCC (49699)	Type c [strain C 9007]	0.016x LoD
	ATCC (9008)	Type d [strain AMC 36-A-6]	0.014x LoD
	ATCC (700223)	Type f [strain GA-1264]	0.016x LoD
	ATCC (49766)	L-378	3x LoD
Listeria monocytogenes	ZeptoMetrix (0801534) [a]	Type 1/2b	1x LoD
	ATCC (19115) [a]	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)	Type 4b	0.03x LoD [c]
	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
Neisseria meningitidis (encapsulated)	ATCC (13090) [a]	Serotype B. M2092 [CIP 104218, L. Cunningham]	1x LoD
	ATCC (35561) [a]	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) [b]	sequence with variant *ctrA* 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
Streptococcus agalactiae	ZeptoMetrix (0801545) [a]	Z019	1x LoD
	ATCC (13813) [a]	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

Pathogen	Source (Catalog ID)	Strain/Serotype	Lowest Concentration Tested
	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
Streptococcus pneumoniae	ZeptoMetrix (0801439) [a]	19F	1x LoD
	ATCC (33400) [a]	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 [Colombia 5-19]	0.03x LoD
	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. Strain [CIP 104225]	3x LoD
	ATCC (BAA-661)	DCC1476 [Sweden 15A-25]	0.03x LoD
Streptococcus pyogenes	ZeptoMetrix (0804351) [a]	Z472; Serotype M1	1x LoD
	ATCC (19615) [a]	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) [a]	Coxsackievirus A16	1x LoD
	ATCC (VR-1801) [a]	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)	A2 Fl [Fleetwood]	3x LoD [c]
	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) [a]	Coxsackievirus B5	1x LoD
	ZeptoMetrix (0810017CF) [a]	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

Pathogen	Source (Catalog ID)	Strain/Serotype	Lowest Concentration Tested
	NCPV (0901047v)	Echovirus 18	3x LoD
	ATCC (VR-41)	Species B, Serotype E-11	3x LoD [c]
Enterovirus C	ATCC (VR-1023) [a]	Coxsackievirus A17, species C. Strain G-12	1x LoD
	ATCC (VR-583) [a]	Coxsackievirus A24. Starin DN-19	1x LoD
	ATCC (VR-850)	Coxsackievirus A21. Strain Kuykendall [V-024-001-012]	3x LoD [c]
	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) [a]	EV 70, species D, strain J670/71	1x LoD
	ATCC (VR-1823) [a]	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
Cryptococcus neoformans	ATCC (MYA-4567) [a]	Serotype D strain WM629, type VNIV	1x LoD
	ATCC (208821) [a]	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 grubiiYL99α	1:100 from stock
Cryptococcus gattii	ATCC (MYA-4094) [a]	Serotype B strain R272, type VGIIb	1x LoD
	ATCC (MYA-4877) [a]	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

Pathogen	Source (Catalog ID)	Strain/Serotype	Lowest Concentration Tested
	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

[a] Strains tested and evaluated during the Limit of Detection studies.
[b] Commercial artificial dsDNA fragment (gBlock) including *ctrA* gene sequence was tested due to unavailability of analytical sample for this variant (GenBank Accession ID HQ156899).
[c] Higher concentration tested to meet 100% detection rate (30xLoD for ATCC (VR-1550, VR-850 and VR-41) and 3xLoD for ZeptoMetrix (0804339)).

Table 4: Inclusivity Strains not detected by the QIAstat-Dx ME Panel

Pathogen	Strain/Serotype
Escherichia coli K1	NCDC Bi 7509-41 Serotype O7:K1(L):NM
Escherichia coli K1	Z136 CTX-M-15
Enterovirus C	CV-A21. Strain H06452 472
Enterovirus C	CV-A21. Strain H06418 508
Streptococcus agalactiae	Serotype III. Typing strain D136C(3) [3 Cole 106, CIP 82.45]

In silico analysis

An *in silico* prediction of the reactivity of all primers-probe oligonucleotide sequences included in the panel against sequences publicly available in databases was performed. The analysis demonstrated a complete coverage for the detection of all main subtypes (species and serotypes) for every On-Panel target organism included in the QIAstat-Dx ME Panel (Table 5).

Table 5: Organisms with predicted reactivity based on *in silico* analysis

Pathogens	Clinically relevant strains/subtypes detected
Neisseria meningitidis (encapsulated)	Encapsulated serotypes (A, B, C, D, E, H, I, K, L, NG, W, W135, X, Y, Z, 29E).
Cryptococcus gattii/ neoformans	Serotype A (*C. neoformans* var neoformans), serotype D (*C. neoformans* var grubii), serotpyes B and C (*C. gattii* including all VGI,VGII, VGIII, VGIV molecular types).
Listeria monocytogenes	Serotypes 1/2a,1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 4e, 7.
Haemophilus influenzae	All encapsulated serotypes (a, b, c, d, e, f) and unencapsulated strains (non-typeable, NTHi) including var. *H. Aegyptus*
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, EV-A120), Enterovirus B (EV-B69, EV-B73 through EV-B75, EV-B79, EV-B80 through EV-B88, EV-B93, EV-B97, EV-B98, EV-B100, EV-B101, EV-B106, EV-B107, EV-B111), Enterovirus C (EV-C96, EV-C99, EV-C102, EV-C104, EV-C105, EV-C109, EV-C116 through EV-C118), Enterovirus D (EV-D68, EV-D70, EV-D94), Poliovirus (PV-1 through PV-3).
Escherichia coli K1	K1 strains

Pathogens	Clinically relevant strains/subtypes detected
Rest of On-Panel organism with no biological subclassification (*S. pneumoniae, S. agalactiae, S. pyogenes*)	All genomic sequences available in databases detected

Analytical Specificity (Exclusivity)

The analytical specificity study was carried out by *in vitro* 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 content (panel exclusivity). The Off-Panel organisms were selected because they are clinically relevant (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.

After testing several On-Panel (13) and Off-Panel (115) strains, the QIAstat-Dx ME Panel demonstrated high specificity levels for all the rtPCR assays included in the device. Additional bioinformatic prediction analysis supported the specificity level, with few exceptions.

In vitro analysis
Samples were prepared by spiking potential cross-reactive organisms into artificial CSF matrix at 10^5 $TCID_{50}$/ml for viral targets, 10^5 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. The On-Panel and Off-Panel organisms tested are shown in Table 6.

All On-Panel pathogens resulted in specific detection, and all Off-Panel pathogens tested showed a negative result and no cross-reactivity was observed in the QIAstat-Dx ME Panel, except for the pathogens shown in Table 7.

Table 6: Off-Panel and On-Panel Analytical strains tested during Exclusivity

Type	Pathogen	Strain/Serotype
On-Panel		
Bacteria	*Escherichia coli* K1	Strain C5 [Bort]; O18ac:K1:H7
Bacteria	*Haemophilus influenzae*	Type e [strain AMC 36-A-7]
Bacteria	*Listeria monocytogenes*	Type 4b. Strain Li 2

Type	Pathogen	Strain/Serotype
On-Panel		
Bacteria	*Neisseria meningitidis*	Serotype Y. M-112 [BO-6]
Bacteria	*Streptococcus pneumoniae*	19F
Bacteria	*Streptococcus agalactiae*	Z019
Bacteria	*Streptococcus pyogenes*	Z472; Serotype M1
Virus	Enterovirus A[1]	A6, species A. Strain Gdula
Virus	Enterovirus B[1]	Coxsackievirus B5
Virus	Enterovirus C[1]	Coxsackievirus A17, species C. Strain G-12
Virus	Enterovirus D[1]	Enterovirus D68. Strain US/MO/14-18947
Yeast	*Cryptococcus neoformans*[1]	WM629 [CBS 10079]
Yeast	*Cryptococcus gattii*[1]	Serotype B strain R272, type VGIIb
Off-Panel		
Virus	Adenovirus A12	Huie
Virus	Adenovirus C2	Adenoid 6 (NIAID 202-001-014)
Virus	Adenovirus D20	A.A
Virus	Adenovirus E4	RI-67
Virus	Adenovirus F41	Tak
Virus	BK polyoma virus	N/A
Virus	Coronavirus 229E	229E
Virus	Coronavirus NL63	NL63 (Amsterdam I)
Virus	Coronavirus OC43	OC43
Virus	Dengue virus (Type 2)	New Guinea C
Virus	Epstein-Barr Virus	B95-8
Virus	Hepatitis B virus (HBV)	N/A
Virus	Hepatitis C virus (HCV)	N/A
Virus	Human herpes virus 7	SB
Virus	Human herpes virus 8	N/A
Virus	Human Immunodeficiency Virus	Quantitative Synthetic Human immunodeficiency virus 1 (HIV-1) RNA
Virus	Human Rhinovirus A1b	2060
Virus	Human Rhinovirus A16	11757
Virus	Human Rhinovirus B3	FEB
Virus	Human Rhinovirus B83	Baylor 7 [V-190-001-021]
Virus	Influenza A H1N1	A/Florida/3/2006

Type	Pathogen	Strain/Serotype
Off-Panel		
Virus	Influenza A H1N1-2009	A/California/08/2009 (H1N1pdm)
Virus	Influenza A H3N2	A/Port Chalmers/1/73
Virus	Influenza B	B/Virginia/ATCC4/2009
Virus	JC polyoma virus	MAD-4
Virus	Measles Virus	Edmonston
Virus	Mumps Virus	Jones
Virus	West Nile Virus	1986
Virus	Parainfluenza virus 2	Greer
Virus	Parainfluenza virus 4	N/A
Virus	Parvovirus B19	B19
Virus	Respiratory Syncytial Virus	A2
Virus	Rotavirus	RRV (Rhesus Rotavirus)
Virus	Rubella Virus	N/A
Virus	St. Louis Encephalitis Virus [1]	Parton
Virus	Cytomegalovirus	Davis
Virus	Herpes simplex virus 1	Macintyre
Virus	Herpes simplex virus 2	HSV-2. (Strain: MS)
Virus	Human herpes virus 6	HHV-6B. (Strain: Z29)
Virus	Human parechovirus	Serotype 3
Virus	Varicella-zoster virus	Ellen
Fungi/parasite	*Candida glabrata*	CBS 138
Fungi/parasite	*Candida krusei*	N/A
Fungi/parasite	*Candida lusitaniae*	Z010
Fungi/parasite	*Candida metapsilosis*	MCO429
Fungi/parasite	*Candida orthopsilosis*	MCO471
Fungi/parasite	*Candida viswanathii*	PK 233 [NCYC 997, pK233]
Fungi/parasite	*Candida parapsilosis*	CBS 604
Fungi/parasite	*Candida tropicalis*	Vitek #8935
Fungi/parasite	*Cryptococcus albidus*	AmMS 228
Fungi/parasite	*Cryptococcus amylolentus*	NRRY Y-7784
Fungi/parasite	*Cryptococcus laurentii*	CBS 139
Fungi/parasite	*Cryptococcus uniguttulatus*	AmMS 234
Fungi/parasite	*Cryptococcus adeliensis* = *Cryptococcus adeliae* = *Naganishia adeliensis*	Cryptococcus adeliae
Fungi/parasite	*Cryptococcus flavescens* = *Papiliotrema flavescens*	Cryptococcus laurentii var. Flavescens (Saito) Lodder et Kreger-van Rij

Type	Pathogen	Strain/Serotype
Off-Panel		
Fungi/parasite	*Cryptococcus wingfieldii = Tsuchiyaea wingfieldii*	OTU 26
Fungi/parasite	*Cryptococcus depauperatus = Aspergillus depauperatus = Filobasidiella depauperate*	K [ARSEF 2058, CBS 7842]
Fungi/parasite	*Filobasidium capsuligenum*	ML-186
Fungi/parasite	*Naegleria fowleri*	Genomic DNA from *Naegleria fowleri*
Fungi/parasite	*Toxoplasma gondii*	Haplogroup 2
Fungi/parasite	*Aspergillus fumigatus*	Z014
Fungi/parasite	*Candida albicans*	CBS 562
Fungi/parasite	*Candida dubliniensis*	Z145
Bacteria	*Bacillus cereus*	Z091
Bacteria	*Citrobacter freundii*	[ATCC 13316, NCTC 9750]
Bacteria	*Corynebacterium striatum*	CDC F6683
Bacteria	*Corynebacterium urealyticus*	3 [Garcia strain]
Bacteria	*Cronobacter (Enterobacter) sakazakii*	CDC 4562-70
Bacteria	*Enterobacter aerogenes*	Z052
Bacteria	*Enterobacter cloacae*	CDC 442-68
Bacteria	*Escherichia coli* (non-K1)	2003-3055
Bacteria	*Escherichia fergusonii*	Z302
Bacteria	*Escherichia hermannii*	CDC 980-72
Bacteria	*Escherichia vulneris*	CDC 875-72
Bacteria	*Haemophilus ducreyi*	DCC1476 [Sweden 15A-25]
Bacteria	*Haemophilus haemolyticus*	NCTC 10659
Bacteria	*Haemophilus parahaemolyticus*	536 [NCTC 8479]
Bacteria	*Haemophilus parainfluenzae*	NCTC 7857
Bacteria	*Klebsiella pneumoniae*	NCTC 9633 [NCDC 298-53, NCDC 410-68]
Bacteria	*Listeria innocua*	SLCC 3379
Bacteria	*Listeria ivanovii*	Li 1979
Bacteria	*Morganella morganii*	AM-15
Bacteria	*Streptococcus salivarius*	C699
Bacteria	*Streptococcus sanguinis*	DSS-10

Type	Pathogen	Strain/Serotype
Off-Panel		
Bacteria	*Streptococcus pseudopneumoniae*	CDC-SS-1757
Bacteria	*Mycoplasma genitalium*	M30
Bacteria	*Neisseria lactamica*	NCDC A7515
Bacteria	*Neisseria mucosa*	AmMS 138
Bacteria	*Neisseria sicca*	AMC 14-D-1
Bacteria	*Neisseria gonorrhoeae*	Z017
Bacteria	*Pantoea agglomerans*	Enterobacter agglomerans
Bacteria	*Proprionibacterium acnes*	NCTC 737
Bacteria	*Proteus mirabilis*	LRA 08 01 73 [API SA, DSM 6674]
Bacteria	*Pseudomonas aeruginosa*	PRD-10 [CIP 103467, NCIB 10421, PCI 812]
Yeast	*Saccharomyces cerevisiae*	NRRL Y-567
Bacteria	*Salmonella bongori*	CIP 82.33
Bacteria	*Salmonella enterica*	CDC K-1891 [ATCC 25928]
Bacteria	*Serratia marcescens*	PCI 1107
Bacteria	*Shigella boydii*	CDC C-123
Bacteria	*Shigella flexneri*	Z046
Bacteria	*Shigella sonnei*	AMC 43-GG9
Bacteria	*Staphylococcus aureus*	FDA 209
Bacteria	*Staphylococcus capitis*	PRA 360 677
Bacteria	*Staphylococcus epidermidis*	FDA strain PCI 1200
Bacteria	*Staphylococcus haemolyticus*	SM 131
Bacteria	*Staphylococcus hominis*	Z031
Bacteria	*Staphylococcus lugdunensis*	LRA 260.05.79
Bacteria	*Staphylococcus saprophyticus*	NCTC 7292
Bacteria	*Streptococcus anginosus*	NCTC 10713
Bacteria	*Streptococcus bovis*	Z167
Bacteria	*Streptococcus dysgalactiae*	Grouping strain C74
Bacteria	*Streptococcus intermedius*	Z126
Bacteria	*Streptococcus oralis*	Z307
Bacteria	*Streptococcus mitis (tigurinus)*	Clinical Isolate
Bacteria	*Streptococcus mutans*	LRA 28 02 81

[1] Enterovirus A, B, C, D and *Cryptococcus neoformans/gattii* are detected by their specific rtPCR assay with no species discrimination.
[2] *N. meningitidis* was tested at 1.00E+05 CFU/mL instead of 1.00E+06 CFU/mL.

Table 7: Samples showing cross-reactivity with the panel

QIAstat-Dx ME Panel Target	Potential cross-reactive organism	Cross reactive concentration
Haemophilus influenzae	*Haemophilus haemolyticus*	≥1.00E+03 CFU/mL
Cryptococcus neoformans/gattii	*Cryptococcus wingfieldii = Tsuchiyaea wingfieldii*	≥1.00E+01 CFU/mL
	Cryptococcus flavescens = Papiliotrema flavescens	≥4.00E+03 CFU/mL
Cryptococcus neoformans/gattii	*Cryptococcus amylolentus*	≥1.00E+01 CFU/mL

In silico analysis

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

The result of the analysis performed for the primer/probe designs included in the QIAstat-Dx ME Panel pointed at 6 potential cross-reactions with Off-Panel targets (listed in Table 8).

Table 8: Samples showing cross-reactivity with the panel

Off-Panel organism	On-Panel signal
*Streptococcus pseudopneumoniae**	*Streptococcus pneumoniae*
*Listeria innocua**	*Listeria monocytogenes*
Haemophilus haemolyticus	*Haemophilus influenzae*
Cryptococcus amylolentus	*Cryptococcus neoformans/gatti*
*Cryptococcus depauperatus**	
Cryptococcus wingfieldii	

**in silico* cross-reactive risk was not confirmed by *in vitro* testing.

Interfering Substances

The effect of potentially interfering substances on the detectability of the QIAstat-Dx ME Panel organisms was evaluated. The substances tested in the study included endogenous as well as exogenous substances that are commonly found and/or introduced into CSF specimens during specimen collection.

The 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 a level predicted to be above the concentration of the substance likely to be found in CSF sample.

Potentially interfering endogenous and exogenous substances have been evaluated and have been confirmed not to interfere with any of the panel target assays at concentrations potentially found in clinical samples (above the concentration of the substance likely to be found in a real CSF specimen). As an exception, bleach was shown to cause inhibition at high concentrations (1% v/v, 0.1% v/v), and showed no interference when tested at 0.01% v/v. Results are provided in Table 9.

Table 9: Final highest concentrations per potentially interfering substance without observable interference

Substance	Concentration Tested	Result
Endogenous substances		
Human Blood	10% (v/v)	No interference
gDNA	20 µg/mL	No interference
D(+)Glucose	10 mg/mL	No interference
L-lactate (Na)	2.2 mg/mL	No interference
Immunoglobulin G (human)	20 mg/mL	No interference
Albumin (human)	30 mg/mL	No interference
Peripheral blood mononuclear cells	10,000 cells/µL	No interference
Exogenous substances		
Chlorhexidine	0.4% (w/v)	No interference
Ethanol	7.0% (v/v)	No interference
Bleach	1.0% (v/v)	Interference
Bleach	0.1% (v/v)	Interference
Bleach	0.01% (v/v)	No interference
Acyclovir	69 µg/mL	No interference
Amphotericin B	5.1 µg/mL	No interference
Ampicillin	210 µg/mL	No interference
Ceftriaxone	840 µg/mL	No interference
Cefotaxime	645 µg/mL	No interference

Microbial Interference

A microbial interference study was conducted to assess the inhibitory effects of select non-target organisms on the ability to detect the QIAstat-Dx ME Panel target organisms. Challenging concentrations (10^5 units/ml for viral targets and 10^6 CFU/mL for bacterial targets) of non-target organisms were individually mixed with artificial CSF matrix containing spiked targeted QIAstat-Dx ME Panel organisms at 3x LoD. Testing was performed in triplicate. All QIAstat-Dx ME Panel organisms were successfully detected

(100% hit rate) when tested in combination with the potentially microbial interferents. See Table 10 for a list of the non-target organisms tested and the result summary.

Table 10: Final highest concentration without observable inhibitory effect

Substance	Supplier / Catalog #	Concentration Tested	Result
Epstein-Barr virus	ZeptoMetrix, 0810008CF	1E+05 cp/mL	No interference
Influenza A H1N1- 2009	ATCC, VR-1895	1E+05 $CEID_{50}$/mL	No interference
Cutibacterium acnes	ATCC, 6919	1E+06 CFU/mL	No interference
Staphylococcus epi-dermidis	ATCC, 14990	1E+06 CFU/mL	No interference
Escherichia coli (non-K1)	ATCC, 25922	1E+06 CFU/mL	No interference
Staphylococcus aureus	ATCC, 29213	1E+06 CFU/mL	No interference
Measles Virus	ATCC, VR-24	1E+05 $TCID_{50}$/mL	No interference

Competitive Inhibition

Combined samples containing a mixture of two different targets spiked at low and high concentrations into artificial CSF were tested. Selection of bacteria, virus, and yeast pathogens and combinations of targets tested was based on clinical relevance.

Clinically relevant co-infection testing demonstrated that when at least two QIAstat-Dx ME Panel pathogens of different concentrations are simultaneously present in one sample, all targets can be detected by the assay. A summary of the final co-infection mixes whereby the High Positive Analyte (HPA) does not inhibit the Low Positive Analyte (LPA) is shown in Table 11.

Table 11: Summary of competitive inhibition testing results

LPA			HPA		
Pathogen	**Concentration**	**Units**	**Pathogen**	**Concentration**	**Units**
Escherichia coli K1	3.30E+02	CFU/mL	*Haemophilus influenzae*	1.00E+06	CFU/mL
Haemophilus influenzae	9.48E+02	CFU/mL	*Escherichia coli K1*	1.00E+06	CFU/mL
Haemophilus influenzae	9.48E+02	CFU/mL	*Streptococcus pneu-moniae*	1.00E+06	CFU/mL
Streptococcus pneu-moniae	6.78E+02	CFU/mL	*Haemophilus influenzae*	1.00E+06	CFU/mL
Listeria monocytogenes	5.58E+03	CFU/mL	*Streptococcus pneu-moniae*	1.00E+06	CFU/mL
Streptococcus pneu-moniae	6.78E+02	CFU/mL	*Listeria monocytogenes*	1.00E+06	CFU/mL
Cryptococcus neo-formans	6.63E+03	CFU/mL	*Streptococcus pneu-moniae*	1.00E+06	CFU/mL
Streptococcus pneu-moniae	6.78E+02	CFU/mL	*Cryptococcus neo-formans*	1.00E+05	CFU/mL
Neisseria meningitidis	3.99E+01	CFU/mL	*Haemophilus influenzae*	1.00E+06	CFU/mL

LPA			HPA		
Pathogen	**Concentration**	**Units**	**Pathogen**	**Concentration**	**Units**
Enterovirus	4.80E+02	TCID50/ mL	*Streptococcus pyogenes*	1.00E+06	CFU/mL
Streptococcus pyogenes	1.71E+03	CFU/mL	Enterovirus	1.00E+05	TCID50/ mL

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. Pathogenic CSF samples with alternating high-positive (10^5–10^6 organism/mL) and negative samples, were conducted 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.

Sample Stability

Sample stability testing demonstrated that the QIAstat-Dx ME Panel assay is capable of processing samples (clinical cerebrospinal fluid specimens) that are stored at different temperatures without affecting the performance.

Sample stability testing demonstrated that CSF specimens may be stored at the conditions listed below.

- Room temperature up to 24 hours at 15-25ºC
- Refrigerated up to 7 days at 2-8ºC

Matrix Equivalency

A matrix equivalency study was conducted to compare the performance of analytical samples prepared in negative clinical CSF matrix to samples prepared in artificial CSF matrix.

A total of 26 pathogen strains (at least two different strains per panel target) were tested in combined samples spiked in clinical true-negative CSF. 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 combined (multiple-spike) sample mixes containing up to 4 organisms per sample in clinical CSF at 5x LoD, 1x LoD and 0.1x LoD concentrations following the Limit of Detection in Combined Samples study. 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 clinical CSF, and a minimum of 10 replicates were tested.

The LoD in clinical sample matrix using combined samples has been established for 26 pathogen strains, and in 18 of those strains the LoD concentration was found to be equivalent to the one established in artificial CSF matrix. Eight (8) pathogen strains were not equivalent and had a new LoD concentration determined in clinical CSF.

Claimed LoD concentrations represent the highest (most concentrated) titer of analyte identified from LoD determination in clinical CSF or artificial CSF.

Precision (Repeatability and Reproducibility)

For the reproducibility assessment, a multi-site scheme was followed by testing both negative and positive samples at three different study sites with varying workflow variables, such as sites, days, instruments, operators and cartridge lots that could have an impact on the precision of the system. Negative samples consisted of artificial CSF. Positive combined samples consisted of artificial CSF spiked with a representative panel of pathogens covering all types of organisms targeted by the QIAstat-Dx ME Panel (i.e., RNA virus, gram (+) bacteria, gram (-) bacteria and yeast) at the limit of detection (1x LoD) and at 3x LoD. For each site, testing was performed across 5 non-consecutive days per mix with 6 replicates per day per mix (leading to a total of 90 replicates per target, concentration, and site), a minimum of 9 different QIAstat-Dx Analyzers per site, and at least 3 operators on each testing day.

Reproducibility testing was designed to evaluate the critical variables that may impact the performance of the QIAstat-Dx ME Panel in the context of its routine and intended use.

For the repeatability study, the same sample panel was tested following a single-site scheme. Repeatability testing was designed to evaluate the precision of a QIAstat-Dx ME Panel Cartridge under similar (intra laboratory) conditions. The Repeatability study was assessed with the same samples used for Reproducibility testing using Site 1.

The Reproducibility and Repeatability studies for the QIAstat-Dx ME Panel demonstrated that no test condition has a specific impact on the test performance or pathogen calling and

confirmed its successful precision since the panel performance or pathogen calling showed to be repeatable along the testing days (results are shown in Table 12 and Table 13).

Table 12: Reproducibility study agreement rate (detected vs. expected) per analyte and the 2-sided 95% Confidence Interval by target for 1x LoD, 3x LoD and Negative samples

Grouping Variable(s)			Proportion		Two-Sided 95% Confidence Limit	
Target	**Concentration**	**Site**	**Fraction**	**Percentage (%)**	**Lower**	**Upper**
Cryptococcus neoformans/gattii	1xLoD	1	30 / 30	100.00	88.43	100.00
		2	30 / 30	100.00	88.43	100.00
		3	30 / 30	100.00	88.43	100.00
		All	90 / 90	100.00	95.98	100.00
	3xLoD	1	30 / 30	100.00	88.43	100.00
		2	30 / 30	100.00	88.43	100.00
		3	30 / 30	100.00	88.43	100.00
		All	90 / 90	100.00	95.98	100.00
Enterovirus	1xLoD	1	30 / 30	100.00	88.43	100.00
		2	30 / 30	100.00	88.43	100.00
		3	30 / 30	100.00	88.43	100.00
		All	90 / 90	100.00	95.98	100.00
	3xLoD	1	30 / 30	100.00	88.43	100.00
		2	30 / 30	100.00	88.43	100.00
		3	30 / 30	100.00	88.43	100.00
		All	90 / 90	100.00	95.98	100.00
Escherichia coli K1	1xLoD	1	30 / 30	100.00	88.43	100.00
		2	30 / 30	100.00	88.43	100.00
		3	30 / 30	100.00	88.43	100.00
		All	90 / 90	100.00	95.98	100.00
	3xLoD	1	30 / 30	100.00	88.43	100.00
		2	30 / 30	100.00	88.43	100.00
		3	30 / 30	100.00	88.43	100.00
		All	90 / 90	100.00	95.98	100.00
Listeria monocytogenes	1xLoD	1	29 / 30	96.67	82.78	99.92
		2	30 / 30	100.00	88.43	100.00
		3	30 / 30	100.00	88.43	100.00
		All	89 / 90	98.89	93.96	99.97
	3xLoD	1	30 / 30	100.00	88.43	100.00
		2	30 / 30	100.00	88.43	100.00
		3	30 / 30	100.00	88.43	100.00
		All	90 / 90	100.00	95.98	100.00

Grouping Variable(s)			Proportion		Two-Sided 95% Confidence Limit	
Target	**Concentration**	**Site**	**Fraction**	**Percentage (%)**	**Lower**	**Upper**
Streptococcus agalactiae	1xLoD	1	30 / 30	100.00	88.43	100.00
		2	30 / 30	100.00	88.43	100.00
		3	30 / 30	100.00	88.43	100.00
		All	90 / 90	100.00	95.98	100.00
	3xLoD	1	30 / 30	100.00	88.43	100.00
		2	30 / 30	100.00	88.43	100.00
		3	30 / 30	100.00	88.43	100.00
		All	90 / 90	100.00	95.98	100.00
n/a	Negative	1	30 / 30	100.00	88.43	100.00
		2	30 / 30	100.00	88.43	100.00
		3	30 / 30	100.00	88.43	100.00
		All	90 / 90	100.00	95.98	100.00

Table 13: Repeatability study agreement rate (detected vs. expected) per analyte and the 2-sided 95% Confidence Interval by target for 1x LoD, 3x LoD and Negative samples

Grouping Variable(s)		Proportion		Two-Sided 95% Confidence Limit	
Target	**Concentration**	**Fraction**	**Percentage (%)**	**Lower**	**Upper**
Cryptococcus neoformans/gattii	1xLoD	60 / 60	100.00	94.04	100.00
	3xLoD	60 / 60	100.00	94.04	100.00
Enterovirus	1xLoD	57 / 60	95.00	86.08	98.96
	3xLoD	60 / 60	100.00	94.04	100.00
Escherichia coli K1	1xLoD	56 / 60	93.33	83.80	98.15
	3xLoD	60 / 60	100.00	94.04	100.00
Listeria monocytogenes	1xLoD	57 / 60	95.00	86.08	98.96
	3xLoD	59 / 60	98.33	91.06	99.96
Streptococcus agalactiae	1xLoD	60 / 60	100.00	94.04	100.00
	3xLoD	60 / 60	100.00	94.04	100.00
Negative	Negative	60 / 60	100.00	94.04	100.00

Performance Characteristics - Clinical Studies

Expected values

In the prospective clinical performance evaluation of the QIAstat-Dx Meningitis / Encephalitis (ME) Panel residual cerebrospinal fluid specimens were collected. Specimens

were tested at 13 geographically diverse clinical sites across 4 countries (3 sites in Europe and 10 sites in USA) from March 2022 to March 2023. The number and percentage of positive results as determined by the QIAstat-Dx ME Panel, stratified by age group are presented in Table 14. 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 analysed in the study (4.3% positivity rate).

Table 14: Table Summary of Prevalence of Target Pathogens, as identified by QIAstat-Dx ME Panel, stratified by age

Pathogen	Overall	<1 year	1-17 years old	18-44 years old	45-64 years old	65-84 years old	>85 years old	Unknown
Bacteria								
Escherichia coli K1	2 (0.1%)	2 (1.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Haemophilus influenzae	7 (0.5%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	5 (1.1%)	1 (0.3%)	0 (0.0%)	0 (0.0%)
Listeria monocytogenes	4 (0.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.5%)	1 (0.3%)	1 (3.2%)	0 (0.0%)
Neisseria meningitidis (encapsulated)	2 (0.1%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	1 (0.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Streptococcus agalactiae	3 (0.2%)	1 (0.6%)	0 (0.0%)	0 (0.0%)	2 (0.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Streptococcus pneumoniae	17 (1.1%)	3 (1.8%)	0 (0.0%)	3 (0.7%)	7 (1.6%)	4 (1.2%)	0 (0.0%)	0 (0.0%)
Streptococcus pyogenes	1 (0.1%)	0 (0.0%)	1 (0.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Virus								
Enterovirus (EV)	23 (1.5%)	12 (7.4%)	3 (2.3%)	5 (1.2%)	1 (0.2%)	1 (0.3%)	0 (0.0%)	1 (50.0%)
Fungi / Yeast								
Cryptococcus gattii / Cryptococcus neoformans (not differentiated)	6 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	5 (1.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Overall Panel Result								
Negative	1462 (95.7%)	145 (89.0%)	124 (96.9%)	407 (97.4%)	419 (94.8%)	336 (98.0%)	30 (96.8%)	1 (50.0%)
Positive	65 (4.3%)	18 (11.0%)	4 (3.1%)	11 (2.6%)	23 (5.2%)	7 (2.0%)	1 (3.2%)	1 (50.0%)

N.B. Total of positive + negative specimens is 1527 due to inclusion of 3 specimens where a QIAstat-Dx result was available but no FilmArray result was available (therefore classed as non-evaluable for the performance analysis).

Clinical Performance

The performance characteristics of the QIAstat-Dx ME Panel was assessed by a multi-centre, observational, prospective and retrospective, clinical performance study, testing fresh and frozen cerebrospinal fluid (CSF) residual specimens obtained by lumbar puncture from patients with signs and symptoms of meningitis and/or encephalitis. The study was conducted at 13 geographically diverse study sites: ten (10) U.S. sites and three (3) European sites. Testing of prospectively collected residual specimens occurred between March 2022 and March 2023. All study sites were hospital-associated or independent clinical diagnostics laboratories that perform routine diagnostics.

Prospective Specimens Testing

A total of 1737 prospective residual CSF specimens were enrolled into the clinical study, of which 205 were withdrawn. The most common reason for specimen withdrawal was ineligibility. Additionally, some prospective samples could not be included in the agreement analysis due to missing data. The final dataset consisted of 1524 prospective specimens of which 552 (36.2%) were frozen before testing and 972 (63.8%) were tested fresh.

Table 15 provides a summary of demographic information for the 1524 specimens included in the prospective study.

Table 15: Demographic data for prospective evaluated specimens

Sample Category	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

Sample Category	Variable	Subgroup	N	%
		Unknown	2	0.1
	Gender	Female	769	50.5
		Male	754	49.5
		Not available	1	0.1

Residual CSF specimens were tested with the QIAstat-Dx ME Panel and two types of comparator methods (an FDA-cleared molecular comparator or two validated end point PCRs followed by bidirectional sequencing (BDS) for selected targets). All targets were compared to the FDA-cleared molecular method except *Streptococcus pneumoniae* and *Streptococcus pyogenes* which were compared against two validated end point PCRs followed by bidirectional sequencing. The standard of care (SoC) testing varied across all sites but included bacterial culture, Laboratory Developed PCR test (LDT), FDA-cleared molecular methods and Cryptococcus antigen screen and culture. Standard of care (SoC) culture results were collected to allow an assessment of clinical sensitivity and specificity and were investigated in cases of discordant result.

Clinical sensitivity or positive percent agreement (PPA) was calculated as 100% x (TP / (TP + FN)). True positive (TP) indicates that both QIAstat-Dx ME Panel and comparator method have a positive result for the specific pathogen. False negative (FN) indicates that the QIAstat-Dx result is negative while the comparator result is positive for the specific pathogen. Clinical Specificity or Negative Percent agreement (NPA) was calculated as 100% x (TN / (TN + FP)). True negative (TN) indicates that both the QIAstat- Dx Panel and the comparator method have negative results for the specific pathogen. False positive (FP) indicates that the QIAstat- Dx Panel result is positive for the specific pathogen, but the comparator result is negative. The two-sided 95% confidence intervals were calculated (Wilson Score Method).

The QIAstat-Dx ME Panel prospective data for positive percent agreement (PPA) and negative percent agreement (NPA) against the comparator methods are presented by analyte in Table 16. Discrepancies between the QIAstat-Dx ME Panel and the comparator methods were investigated and discrepancy investigations are footnoted in Table 16 below.

Table 16: Prospectively Collected Clinical Specimens (fresh and frozen) Performance Agreement (PPA and NPA) between QIAstat-Dx ME Panel and Comparator Method by Target

		Positive Percent Agreement			Negative Percent Agreement		
Pathogen	**Sample Category**	**TP/TP+FN**	**%**	**95% CI***	**TN/TN+FP**	**%**	**95% CI***
Bacteria							
Escherichia coli K1[a]	Prospective Fresh	2 / 3	66.7	20.8-93.9	969 / 969	100.0	99.6-100.0
	Prospective Frozen	0 / 1	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

		Positive Percent Agreement			Negative Percent Agreement		
Pathogen	**Sample Category**	**TP/TP+FN**	**%**	**95% CI***	**TN/TN+FP**	**%**	**95% CI***
Bacteria							
Haemophilus influenzae[b]	Prospective Fresh	0 / 1	0.0	0.0-79.3	970 / 971	99.9	99.4-100.0
	Prospective Frozen	4 / 4	100.0	51.0-100.0	546 / 548	99.6	98.7-99.9
	Overall	4 / 5	80.0	37.6-96.4	1516 / 1519	99.8	99.4-99.9
Listeria monocytogenes[c]	Prospective Fresh	1 / 1	100.0	20.7-100.0	971 / 971	100.0	99.6-100.0
	Prospective Frozen	3 / 4	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
Neisseria meningitidis (encapsulated)[d]	Prospective Fresh	1 / 1	100.0	20.7-100.0	971 / 971	100.0	99.6-100.0
	Prospective Frozen	0 / 0	N/A	N/A	551 / 552	99.8	99.0-100.0
	Overall	1 / 1	100.0	20.7-100.0	1522 / 1523	99.9	99.6-100.0
Streptococcus agalactiae	Prospective Fresh	2 / 2	100.0	34.2-100.0	970 / 970	100.0	99.6-100.0
	Prospective 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
Streptococcus pneumoniae[e]	Prospective Fresh	1 / 1	100.0	20.7-100.0	845 / 848	99.6	99.0-99.9
	Prospective Frozen	7 / 7	100.0	64.6-100.0	515 / 517	99.6	98.6-99.9
	Overall	8 / 8	100.0	67.6-100.0	1360 / 1365	99.6	99.1-99.8
Streptococcus pyogenes	Prospective Fresh	0 / 0	N/A	N/A	778 / 778	100.0	99.5-100.0
	Prospective 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
Virus							
Enterovirus (EV)[f]	Prospective Fresh	18 / 20	90.0	69.9-97.2	951 / 952	99.9	99.4-100.0
	Prospective 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
Fungi / Yeast							
Cryptococcus gattii / *Cryptococcus neoformans* (not differentiated)[g]	Prospective Fresh	2 / 5	40.0	11.8-76.9	965 / 967	99.8	99.2-99.9
	Prospective 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

[a] For the prospective fresh *Escherichia coli K1* discordant sample, no organisms were detected with resolution method PCR/BDS. For the frozen *Escherichia coli* K1 discordant sample no organisms were detected with bacterial culture.
[b] For the prospective fresh *Haemophilus influenzae* discordant sample, no organisms were detected by the SoC bacterial culture and resolution 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 resolution method was also negative. No additional testing results associated with the final frozen false positive sample were available.
[c] For the frozen *Listeria monocytogenes* discordant sample the negative result was confirmed positive with SoC culture and LDT result was positive.
[d] For the frozen *Neisseria meningitidis* (encapsulated) sample, no organisms were detected by SoC culture and LDT resolution testing with PCR/BDS also returned a negative result for this sample.
[e] 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.
[f] 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.
[g] 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.

Archived Specimens Testing

Several analytes were either not encountered or had a low prevalence in the prospective arm of the study, so an effort was made to collect and test as many archived samples (positive targeted archived specimens) as possible for all targets within the panel. A total of 195 retrospective archived specimens were enrolled into the study. One hundred and fifty-four (154) archived specimens were excluded from the analysis as positivity was not confirmed by the comparator method. A total of 41 evaluable archived specimens were used in the analysis to support the QIAstat-Dx ME Panel performance evaluation and Table 17 provides a summary of demographic information for the archived specimens.

Table 17: Demographic Summary of Evaluable Archived Specimens for QIAstat-Dx ME Panel Clinical Evaluation

Sample Category	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 QIAstat-Dx ME Panel retrospective archived specimen data in positive percent agreement and negative percent agreement against the comparator methods are presented by analyte in Table 18.

Table 18: Archived Clinical Performance Agreement (PPA) between QIAstat-Dx ME Panel and Comparator Method by Target*

	Positive Percent Agreement			Negative Percent Agreement		
Pathogen	**TP/TP+FN**	**%**	**95% CI**	**TN/TN+FP**	**%**	**95% CI**
Overall Panel	41 / 41	100.0	91.4-100.0	314 / 314	100.0	98.8-100.0
Bacteria						
Escherichia coli K1	2 / 2	100.0	34.2-100.0	39 / 39	100.0	91.0-100.0
Haemophilus influenzae	6 / 6	100.0	61.0-100.0	35 / 35	100.0	90.1-100.0
Listeria monocytogenes	0 / 0	N/A	N/A	41 / 41	100.0	91.4-100.0
Neisseria meningitidis (encapsulated)	3 / 3	100.0	43.9-100.0	38 / 38	100.0	90.8-100.0
Streptococcus agalactiae	9 / 9	100.0	70.1-100.0	32 / 32	100.0	89.3-100.0
Streptococcus pneumoniae	4 / 4	100.0	51.0-100.0	18 / 18	100.0	82.4-100.0
Streptococcus pyogenes	0 / 0	N/A	N/A	23 / 23	100.0	85.7-100.0
Virus						
Enterovirus (EV)	9 / 9	100.0	70.1-100.0	32 / 32	100.0	89.3-100.0
Fungi / Yeast						
Cryptococcus gattii / Cryptococcus neoformans (not differentiated)	8 / 8	100.0	67.6-100.0	33 / 33	100.0	89.6-100.0

*Please note that due to volume constraints, not all targets have the same number of specimens tested.

For each analyte where archived samples were available, a PPA of 100% was achieved.

Co-Infections

No specimens with multiple detections were identified by the QIAstat-Dx ME Panel during the performance evaluation.

Clinical sensitivity and specificity determined against culture

The performance measure of sensitivity and specificity was calculated only for bacterial and fungi / yeast analytes for which the gold-standard CSF culture result was available in the standard of care for the specimen. This data was used in additional performance calculations outlined in Table 19 and Table 20.

Table 19: Bacterial Culture comparison for diagnostic sensitivity and specificity by sample category

		Sensitivity (compared to culture)			Specificity (compared to culture)		
Pathogen	**Sample Category**	**TP/TP+FN**	**%**	**95% CI**	**TN/TN+FP**	**%**	**95% CI**
Bacteria							
Escherichia coli K1	Archived	1 / 1	100.0	20.7-100.0	10 / 11	90.9	62.3-98.4
	Prospective Fresh	1 / 2	50.0	9.5-90.5	760 / 760	100.0	99.5-100.0
	Prospective Frozen	0 / 0	N/A	N/A	343 / 343	100.0	98.9-100.0
Haemophilus influenzae	Archived	1 / 1	100.0	20.7-100.0	10 / 11	90.9	62.3-98.4
	Prospective Fresh	0 / 0	N/A	N/A	761 / 762	99.9	99.3-100.0
	Prospective Frozen	3 / 3	100.0	43.9-100.0	339 / 340	99.7	98.4-99.9
Listeria monocytogenes	Archived	0 / 0	N/A	N/A	12 / 12	100.0	75.8-100.0
	Prospective Fresh	1 / 1	100.0	20.7-100.0	761 / 761	100.0	99.5-100.0
	Prospective Frozen	2 / 3	66.7	20.8-93.9	340 / 340	100.0	98.9-100.0
Neisseria meningitidis (encapsulated)	Archived	2 / 2	100.0	34.2-100.0	9 / 10	90.0	59.6-98.2
	Prospective Fresh	0 / 0	N/A	N/A	761 / 762	99.9	99.3-100.0
	Prospective Frozen	0 / 0	N/A	N/A	342 / 343	99.7	98.4-99.9
Streptococcus agalactiae	Archived	0 / 0	N/A	N/A	12 / 12	100.0	75.8-100.0
	Prospective Fresh	1 / 1	100.0	20.7-100.0	760 / 761	99.9	99.3-100.0
	Prospective Frozen	1 / 1	100.0	20.7-100.0	342 / 342	100.0	98.9-100.0
Streptococcus pneumoniae	Archived	0 / 0	N/A	N/A	11 / 12	91.7	64.6-98.5
	Prospective Fresh	0 / 0	N/A	N/A	757 / 762	99.3	98.5-99.7
	Prospective Frozen	3 / 3	100.0	43.9-100.0	339 / 340	99.7	98.4-99.9
Streptococcus pyogenes	Archived	0 / 0	N/A	N/A	12 / 12	100.0	75.8-100.0
	Prospective Fresh	0 / 0	N/A	N/A	761 / 762	99.9	99.3-100.0
	Prospective Frozen	0 / 0	N/A	N/A	343 / 343	100.0	98.9-100.0

Cryptococcus was also calculated in comparison to fungal culture performed as a standard of care diagnostic at clinical testing sites. For data that were available, QIAstat-Dx ME Panel performance is summarized in Table 20.

Table 20: Fungal Culture Comparison for diagnostic sensitivity and specificity by sample category

		Positive Percent Agreement			Negative Percent Agreement		
Pathogen	**Sample Category**	**TP/TP+FN**	**%**	**95% CI**	**TN/TN+FP**	**%**	**95% CI**
Fungi / Yeast							
Cryptococcus gattii / *Cryptococcus neoformans* (not differentiated)	Archived	2 / 2	100.0	34.2-100.0	1 / 1	100.0	20.7-100.0
	Prospective Fresh	1 / 1	100.0	20.7-100.0	129 / 131	98.5	94.6-99.6
	Prospective Frozen	0 / 0	N/A	N/A	24 / 24	100.0	86.2-100.0

Validity of results

The failure rate for initial clinical specimens tests for prospective fresh was 2.7% (26/977), for prospective frozen was 1.3% (7/555) for prospective frozen and for archived samples was 1.7% (3/176). Those failures consisted of 5 instrument errors and 2 invalid results, and 29 were other run failures. All specimens except 5 (3 prospective fresh and 2 prospective frozen) were retested and were successful after retest, yielding a final success rate of 99.7% for prospective fresh, 99.6% for prospective frozen and 100.0% for archived samples. The failure rates breakdown due to instrument, invalid results and other run failures is summarized in Table 21. Withdrawn specimens were not included in the validity assessment; however some prospective samples which were excluded from the agreement analysis were included for the validity assessment (3 prospective fresh, 5 prospective frozen and 135 archive specimens).

Table 21: Summary of the Number of Samples with Failed Test Results (Initial and Final)

		Initial Runs		Final Runs (after repeats)	
Sample Category	**Failure Reason**	**N/Total**	**%**	**N/Total**	**%**
Prospective Fresh	**Invalid***	0 / 977	0.0	0 / 977	0.0
	Instrument	3 / 977	0.3	0 / 977	0.0
	Other**	23 / 977	2.4	3 / 977	0.3
Prospective Frozen	**Invalid**	0 / 555	0.0	0 / 555	0.0
	Instrument	1 / 555	0.2	0 / 555	0.0
	Other	6 / 555	1.1	2 / 555	0.4
Archived	**Invalid**	2 / 176	1.1	0 / 176	0.0
	Instrument	1 / 176	0.6	0 / 176	0.0
	Other	0 / 176	0.0	0 / 176	0.0

*Internal Control failures with at least one analyte detected and the other analytes reported as 'invalid'
** Run failures related to workflow checkpoints.

Contrived Specimens Testing

Contrived specimens testing was conducted to support the nine (9) targets on the panel as there were insufficient positive specimens obtained from both prospective and archived collection efforts. Contrived specimens were prepared by spiking five different quantified strains representative of the genetic diversity of each pathogen. For each pathogen, the LoD concentration was manufactured at 2x (at least 50%) and 5x LoD spiked into screened individual unique samples of negative CSF. Contrived specimens were tested alongside negative specimens in a blinded manner. The results are summarized in Table 22.

Table 22: Overall Proportion of Positive Results Post Exclusions or withdrawals of Contrived Positive Samples for Each Target

Classification (genome type)	Pathogen	Concentration Level	Frequency of Positive Results	Proportion (%) of Positive Results	Lower 95% Confidence Limit	Upper 95% Confidence Limit
Bacteria	*Escherichia coli K1*	2xLoD	48 / 48	100.0	92.6	100.0
		5xLoD	37 / 37	100.0	90.6	100.0
		Total	85 / 85	100.0	95.7	100.0
	Haemophilus influenzae	2xLoD	57 / 57	100.0	93.7	100.0
		5xLoD	36 / 36	100.0	90.4	100.0
		Total	93 / 93	100.0	96.0	100.0
	Listeria monocytogenes	2xLoD	47 / 49	95.9	86.3	98.9
		5xLoD	38 / 38	100.0	90.8	100.0
		Total	85 / 87	97.7	92.0	99.4
	Neisseria meningitidis (encapsulated)	2xLoD	46 / 48	95.8	86.0	98.8
		5xLoD	39 / 40	97.5	87.1	99.6
		Total	85 / 88	96.6	90.5	98.8
	Streptococcus agalactiae	2xLoD	49 / 49	100.0	92.7	100.0
		5xLoD	39 / 39	100.0	91.0	100.0
		Total	88 / 88	100.0	95.8	100.0
	Streptococcus pneumoniae	2xLoD	55 / 57	96.5	88.1	99.0
		5xLoD	39 / 39	100.0	91.0	100.0
		Total	94 / 96	97.9	92.7	99.4
	Streptococcus pyogenes	2xLoD	47 / 49	95.9	86.3	98.9
		5xLoD	40 / 40	100.0	91.2	100.0
		Total	87 / 89	97.8	92.2	99.4
Virus	Enterovirus (EV)	2xLoD	48 / 49	98.0	89.3	99.6
		5xLoD	39 / 39	100.0	91.0	100.0
		Total	87 / 88	98.9	93.8	99.8
Fungi / Yeast	*Cryptococcus gattii / Cryptococcus neoformans* (not differentiated)	2xLoD	41 / 41	100.0	91.4	100.0
		5xLoD	38 / 38	100.0	90.8	100.0
		Total	79 / 79	100.0	95.4	100.0

Target Proportion of Positive Result ≥95% was achieved for all prepared contrived samples 2xLoD and 5xLoD in all tested analytes.

Conclusions

The QIAstat-Dx ME Panel is substantially equivalent to the to the predicate device, FilmArray Meningitis/Encephalitis (ME) Panel.