

**510(k) De Novo
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE**

A. 510(k) Number: K061062

B. Purpose for Submission: Marketing authorization of new device

C. Measurand: Enterovirus (EV) RNA (enterovirus genome 5' untranslated region (UTR) between nucleotide 452 and 596)

D. Type of Test: Nucleic acid amplification assay system, automated

E. Applicant: Cepheid

F. Proprietary and Established Names: Xpert EV™

G. Regulatory Information:

1. Regulation section: 21 CFR 866.3225
2. Classification: Class II (de novo)
3. Product code: OAI (Nucleic acid amplification assay system, enterovirus (EV) RNA in cerebrospinal (CSF) specimens, direct specimen test)
4. Panel: 83

H. Intended Use:

1. Intended use(s):

The Cepheid® Xpert EV assay is a reverse transcription polymerase chain reaction (RT-PCR) using the GeneXpert® Dx System for the presumptive qualitative detection of enterovirus (EV) RNA in cerebrospinal fluid (CSF) specimens from individuals with signs and symptoms of meningitis. This test, in conjunction with other laboratory results and clinical information, may be used as an aid in the laboratory diagnosis of enterovirus infection in patients with a clinical suspicion of meningitis or meningoencephalitis.

Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients.

CAUTION: The results obtained with the Xpert EV assay should be used only as an adjunct to clinical observation and other information available to the physician. Positive Xpert EV results do not rule out other causes of meningitis, including bacteria, mycobacteria, other viruses (e.g. herpes family viruses, arboviruses, mumps virus, etc) and fungi.

2. Indications for use: Same as intended use

3. Special conditions for use statement(s):

Prescription Use Only

4. Special instrument requirements: GeneXpert Dx System (instrument, computer, barcode wand reader)

I. Device Description:

The Xpert EV assay is designed to detect enterovirus (EV) RNA (enterovirus genome 5' untranslated region (UTR) between nucleotide 452 and 596) in CSF samples from patients exhibiting meningitis like symptoms. The assay includes reagents, primers, and probes for the simultaneous detection of nucleic acid from the target EV and the sample-processing control/internal control (SPC/IC). The assay includes the SPC/IC to verify adequate processing of the target virus and monitors the presence of inhibitors in the RT-PCR assay to avoid a false negative result. The assay also includes a probe check control to verify reagent rehydration, probe integrity, and reaction-tube filling in the cartridge.

The assay is run on the GeneXpert Dx System. The GeneXpert Dx System automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence using real-time PCR and RT-PCR assays. The system consists of an instrument, personal computer, and preloaded software for running tests on collected samples and viewing the results. The system requires the use of single-use disposable GeneXpert cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is eliminated. The above described sample-processing control/internal control (SPC/IC) is named CIC in the GeneXpert Dx System software.

To run a test, the CSF sample and four reagents are transferred into designated chambers of the Xpert EV cartridge. The GeneXpert Dx System performs sample preparation by lysing the virus and SPC (armored pseudovirus RNA), binding the RNA to the capture matrix, and eluting the RNA. The RNA is mixed with dry RT reagents and transferred into the reaction tube for preparation of cDNA. The cDNA is then mixed with dry PCR reagents and transferred into the reaction tube for real-time PCR and detection. The EV primers and probe amplify and detect a consensus region of the enterovirus 5' untranslated region (UTR). The test takes approximately 2.5 hours.

Xpert EV™ Reagent Kit and Xpert EV™ Self-contained Cartridge



GeneXpert Dx System



J. Substantial Equivalence Information:

1. Predicate device name(s): None
2. Predicate 510(k) number(s): None
3. Comparison with predicate: Not applicable

K. Standard/Guidance Document Referenced (if applicable):

1. A special control guidance document will be promulgated.

2. CDRH Draft Guidance for Industry and FDA staff titled: *Nucleic Acid Based In Vitro Diagnostic Devices for Detection of Microbial Pathogens*, December 8, 2005.

L. Test Principle:

The Xpert EV Test uses real-time PCR adapted to an automated instrument system that integrates sample processing (lysis and RNA purification) in a cartridge that contains freeze-dried PCR reagents and process controls for real-time PCR detection. This integration of nucleic acid extraction reduces labor-intensive steps requiring specially trained personnel and also reduces the likelihood of cross-contamination and technician-dependent variability. The instrument moves the sample and reagents to and from different chambers within the Xpert EV™ cartridge, automatically performing sample preparation by lysing the virus and the SPC/IC, binding the RNA to the capture matrix, and eluting the RNA.

The Xpert EV Target region is a 5' untranslated (UTR) region of the enterovirus genome RNA between nucleotide 452 and 596). The primers and probe sequences used in the Xpert EV assay are designed to these consensus regions and are essentially similar to those developed by Rotbart (J. Clin. Microbiol., **28**, 438-442, 1990; US patent 5075212). The resulting amplicon is 146 bp. Probes are labeled with FAM (5') and QSY7 quencher (3'). Note: Primers are 19 mer (Tm 67.6, 58% GC) and 20 mer (63.4 Tm, 45 %GC) while the EV probe is 25 mer (74.9 Tm, 52 %GC). The reverse transcribing parameters are 30 min at 50° followed by 5 min at 93° and the cycling parameters are 15 min at 95° (denaturation), followed by 45 cycles of 20s at 95°C, 30s at 58°C, and 30s at 73°C.

The GenXpert® instrument has 4 modules, each with an I-Core for thermal cycling and real-time detection of PCR amplicons. Each of the 4 modules is controlled independently, allowing for specimens to be loaded and tested when received, rather than batching. Each of the 4 modules in addition to the I-Core, has a syringe drive for aspirating and dispensing fluids, a valve drive for chamber access, and an ultrasonic horn for lysis. Prior to operation, a self-test verifies heater, fan and optics functionality, while the syringe drive, valve and ultrasonic horn current are continuously checked. The GenXpert can simultaneously detect signal from up four different spectral bands.

Each I-core has two ceramic plates to assure temperature uniformity and rapid heat transfer. Firmware controls the temperature inside by moving ambient air across the heater plates. Optics consist of a 4-color excitor module (high intensity LEDs to excite reporter dye molecules) and a 4-color detector module (silicon photodetectors and filters to detect the 4 spectral bands). Calibration and data analysis algorithms compensate for spectral overlap. Thermal reaction chamber thermistors are calibrated to $\pm 0.50^{\circ}\text{C}$ and calibration coefficients correct for errors in raw thermistor reads (stored in firmware). Individual dye-oligos signals are corrected using spectral characteristics of pure dye-oligos after subtracting raw signal produced by a reaction tube alone.

For the EV application, three spectral signals are processed (separate dyes tagged to oligonucleotide probes for the SPC, IC, and the EV). Two of the 3 signals are from fixed components within the reagents, with one from unknown. Output results are color coded (red-positive; green-negative; gray-invalid; gold-error); tabular and graphic formats are accessible by password.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility was assessed in a multi-center, blinded study using a precision panel consisting of four specimens. Three sites tested each panel three times per day over 10 testing days, for a total of 90 results per panel specimen. The precision panel consisted of a negative sample and three positive samples, each with a specific EV serotype spiked into synthetic CSF at a concentration close to the limit of detection.

Table 1. Summary of first reproducibility study results.

No. of specimens correctly classified					Between Day		Between Site		Total	
Serotype (TCID ₅₀ /mL)	Site 1	Site 2	Site 3	Mean EV Ct	SD	%CV	SD	%CV	SD	%CV
Negative	30/30	30/30	30/30							
CVA6 (134)	30/30	30/30	29/29*	35.0	0.343	0.98%	0.175	0.50%	1.101	3.15%
CVA9 (320)	30/30	30/30	30/30	34.4	0	0.00%	0	0.00%	0.61	1.77%
CVA17 (3)	30/30	30/30	29/29*	33.8	0	0.00%	0	0.00%	0.414	1.22%
Total	120/	120/	118/							
Agreement	120	120	118							
%	100.00	100.00	100.00							
Agreement	%	%	%							

* Two samples at site 3 did not give any GeneXpert result

In order to further stress the system a second study was performed. A reproducibility study was conducted over four different days on 31 GeneXpert instruments and 121 ICORE modules. Two representative whole virus subtypes (i.e., Coxsackievirus CVA9 and Enterovirus EV70) were spiked into human negative CSF to create simulated specimens at both 2 x LoD and 4 x LoD. Negative samples were tested 20 times each day whereas 2 positive samples at 2 different concentrations were tested 5 times per day. Table 2 shows the average Ct values for each concentration and the associated standard deviations and coefficients of variation for each day.

Table 2. Summary of second reproducibility study results.

The level of agreement, the average Ct values for each concentration, the associated standard deviations and percent coefficient of variation for each day are shown in the table below.

Specimen ID		Total Agreement - Ct Results					% Total Agreement
		Day 1	Day 2	Day 3	Day 4	All Days	
Negative	Total Agreement	20/20	18/18 ^A	20/20	20/20	78/78	100%
	Average	NA	NA	NA	NA	NA	
	SD	NA	NA	NA	NA	NA	
	% CV	NA	NA	NA	NA	NA	
CA9 2X LOD	Total Agreement	4/5 ^B	5/5	4/5 ^B	5/5	18/20	90%
	Average	36.65	36.54	36.53	36.54	36.56	
	SD	0.56	0.46	0.21	0.69	0.48	
	% CV	1.53%	1.26%	0.57%	1.89%	1.31%	
CA9 4X LOD	Total Agreement	5/5	5/5	5/5	4/4 ^C	19/19	100%
	Average	34.98	35.56	35.52	35.03	35.28	
	SD	0.53	0.67	0.7	0.3	0.6	
	% CV	1.52%	1.88%	1.97%	0.86%	1.70%	
EV70 2X LOD	Total Agreement	5/5	5/5	5/5 ^D	5/5	20/20	100%
	Average	37.38	37.3	37.55	36.88	37.2	
	SD	1.78	0.74	2.01	0.81	1.3	
	% CV	4.76%	1.98%	5.35%	2.20%	3.49%	
EV70 4X LOD	Total Agreement	5/5	5/5	5/5	5/5	20/20	100%
	Average	36.50	36.60	36.12	35.94	36.29	
	SD	0.58	0.97	0.29	0.84	0.72	
	% CV	1.59%	2.65%	0.80%	2.34%	1.98%	
No. of Instruments used		10	11	10	10	31	
No of Modules used		40	41	41	40	121	

^A Total runs = 21, 2-No Result, 1-Invalid

^B Total runs = 5, 1 negative instead of positive result

^C Total runs = 5, 1-Invalid

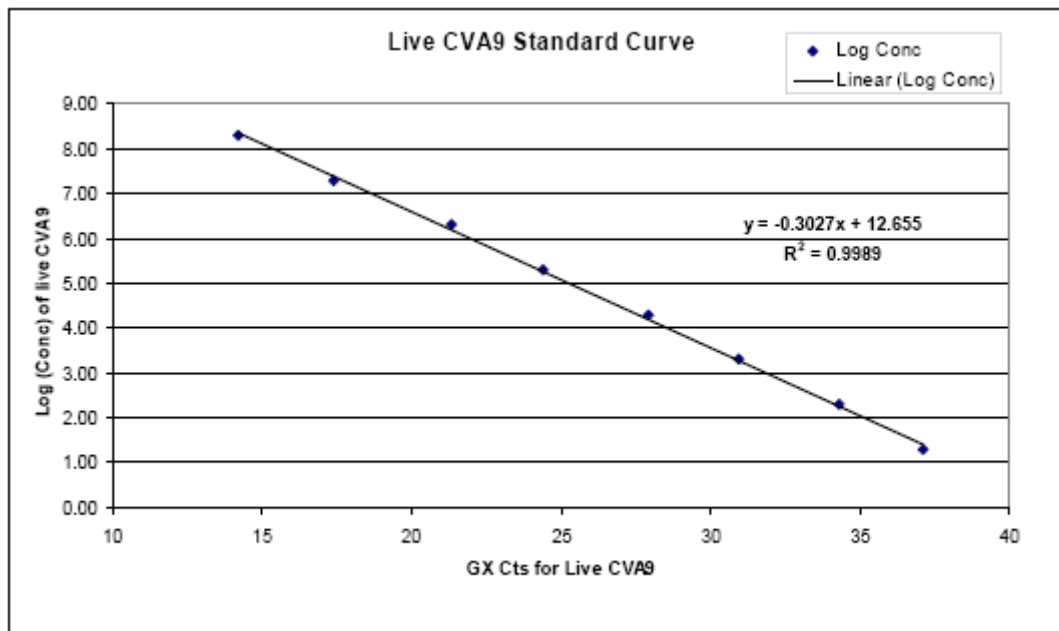
^D Total runs = 6, 1-No Result

Of the total samples tested there were two samples with “Invalid” and three samples with “No Result” by instrument software control definitions. Of the 157 reportable results, 155 were correctly scored.

b. Linearity/assay reportable range:

A stock concentration of CVA9 (2e9 TCID₅₀/mL) was serially diluted in EV Negative synthetic CSF sample and loaded directly into each cartridge with appropriate sample preparation reagents. Duplicates at each dilution were tested. The GeneXpert EV assay detects serotype CVA9 over a dynamic range of 8 logs. The assay is linear over this range with an R² value of 0.9989. The reported Ct range is shown below.

Dynamic Range Standard Curve



c. *Traceability, Stability, Expected Values (controls, calibrators, or methods):* Not applicable

d. *Detection limit:*

The analytical sensitivity, or limit of detection (LoD), is defined as the lowest concentration, or amount of an analyte that has been demonstrated by laboratory analysis to be reproducibly distinguished from a negative sample at a 95% confidence level. The dilutions were made in pooled EV negative human sample. For statistical confidence determination of the LoD, replicates of 20, along with 20 EV negative samples, were run. The samples run were Coxsackievirus A6 (CVA6), Coxsackievirus A9 (CVA9), Coxsackievirus A17 (CVA17), Enterovirus 70 (EV70), and Poliovirus 1 (PV1). Not all 63 serotypes were run in statistically significant numbers, since the primer and probe binding sites are conserved across all serotypes and the amplicon length is the same for all serotypes, so it would be expected that the amplification efficiency is the same for all serotypes. The five serotypes indicated above were selected to represent each of the enterovirus species CVA6 (A), CVA9 (B), CVA17 (C), EV70 (D) and PV1 (poliovirus).

Table 3. Limit of detection for 5 serotypes.

The LoD of the 5 serotypes, one from each of the enterovirus species are shown in the table below.

Serotype	Limit of Detection (TCID ₅₀ /mL)
CVA9 20:	80.0
EV70:	1.3
PV1:	4.0
CVA17:	1.0
CVA6:	33.0

Analytical Reactivity/Enterovirus Serotype testing

A total of 60 enterovirus serotypes were tested with the Xpert EV assay. Dilutions of the viral stock were run in replicates of 3 for each serotype at the presumed LoD. The dilutions were made in pooled EV negative human sample.

Table 4. Estimated analytical sensitivity.

Sixty of the serotypes were tested and the estimated TCID₅₀/ml that these serotypes can be detected were shown in Table 4 below.

Table 4 Estimated Analytical Sensitivity

Species	Serotype	Estimated TCID ₅₀ /mL
A	Coxsackie A3	5.01
A	Coxsackie A5	12.59
A	Coxsackie A6	12.59
A	Coxsackie A7	3.33
A	Coxsackie A10	2.81
A	Coxsackie A12	19.95
A	Coxsackie A14	0.10
A	Coxsackie A16	0.002
A	EV 71	0.16
B	Coxsackie A9	20.00
B	Coxsackie B1	4.00
B	Coxsackie B2	0.20
B	Coxsackie B3	0.028
B	Coxsackie B4	0.40
B	Coxsackie B5	0.04
B	Coxsackie B6	0.01
B	Echo 1	0.10
B	Echo 2	0.032
B	Echo 3	200.00
B	Echo 4	0.00032
B	Echo 5	0.032
B	Echo 6	200.00
B	Echo 7	2.00
B	Echo 8	0.10
B	Echo 9	2.00
B	Echo 11	40.00
B	Echo 12	1.58
B	Echo 14	0.0005
B	Echo 15	0.0032
B	Echo 16	0.0005
B	Echo 17	0.05
B	Echo 18	0.0002
B	Echo 19	2.51
B	Echo 20	0.032
B	Echo 21	1.00
B	Echo 24	0.02
B	Echo 25	0.50
B	Echo 26	0.032
B	Echo 27	0.00032
B	Echo 29	5.01
B	Echo 30	0.01
B	Echo 31	0.0032
B	Echo 32	0.10
B	Echo 33	0.05
B	EV 69	0.0002
C	Echo 13	0.01
C	Coxsackie A11	0.11
C	Coxsackie A13	13.27
C	Coxsackie A15	0.00
C	Coxsackie A17	1.58
C	Coxsackie A18	0.02
C	Coxsackie A19	0.03
C	Coxsackie A20	0.002
C	Coxsackie A21	0.03
C	Coxsackie A22	0.02
C	Coxsackie A24	0.10
D	EV 68	199.53
D	EV 70	2.00
Poliovirus	Poliovirus 1	2.00
Poliovirus	Poliovirus 2	0.40
Poliovirus	Poliovirus 3	20.00

e. *Analytical specificity:*

Nucleic acids from $1-2 \times 10^8$ cells of EBV, HSV-1, HSV-2, HHV-6, HHV-7, Adenovirus-2, Measles, Mumps, Parainfluenza 1-3, Influenza A, Influenza B, VZV, CMV, Group B *Streptococcus*, *Haemophilus influenzae* B, *H. influenzae* non-B, *Escherichia coli*, *Neisseria meningitidis*, *Citrobacter freundii*, and *Citrobacter koseri* were isolated with commercially available isolation kits and assayed in the Xpert. In addition, the specificity evaluation was repeated with “Whole Organisms” at titer levels much greater than those found typically in CSF infected with the respective organisms. The Xpert EV assay did not generate any detectable amplicons when “Whole Organisms” of the above listed pathogens were processed through the Xpert EV cartridge.

Also, high concentrations of both intact white blood cells and total RNA isolated from white blood cells were used in assays with the Xpert EV assay. There was no cross-hybridization of purified human genomic RNA from WBC with the primer and probe sequences used in the Xpert EV assay.

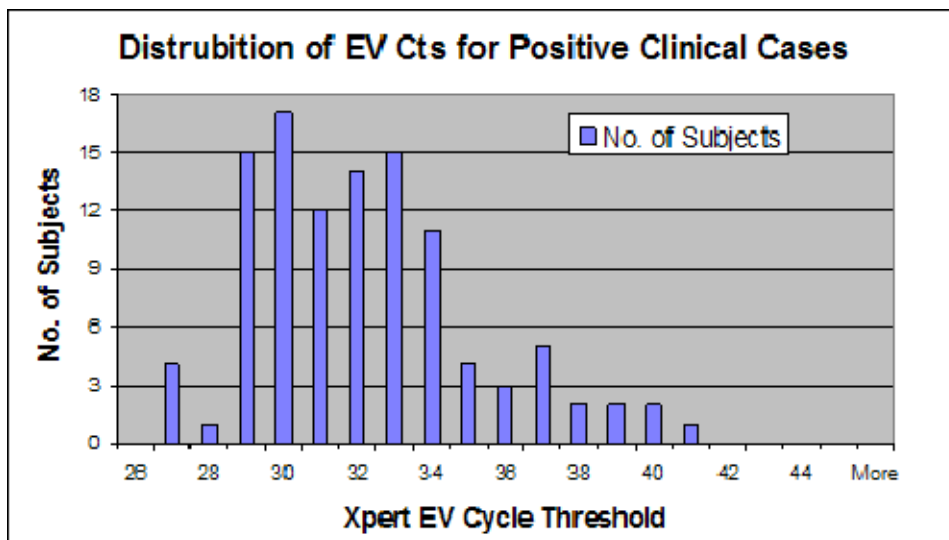
Table 5. Analytical specificity for the Xpert EV.

Whole organisms were tested for specificity in the Xpert EV assay and the concentrations of the organisms tested were shown in the table below.

Organism	No. organisms/test
HHV6	3.1e6 particles
HHV7	1.4e7 particles
CMV	700 TCID ₅₀
EBV	140 TCID ₅₀
HSV 1	1.4e5 TCID ₅₀
HSV 2	1.4e5 TCID ₅₀
ADV 2	1.4e12 TCID ₅₀
Measles	700 TCID ₅₀
Mumps	1.4e4 TCID ₅₀
Parainfluenza 1	1.4e3 TCID ₅₀
Parainfluenza 2	7e3 TCID ₅₀
Parainfluenza 3	1.4e4 TCID ₅₀
Influenza A	3.5e4 TCID ₅₀
Influenza B	3.5e4 TCID ₅₀
VZV	14 TCID ₅₀
Group B Streptococcus	7e6 cells
<i>H. influenzae</i> B	7e6 cells
<i>H. influenzae</i> NonB	7e5 cells
<i>E.coli</i>	7e6 cells
<i>N. meningitidis</i>	7e6 cells
<i>C. freundii</i>	7e6 cells
<i>C. koseri</i>	7e6 cells

f. **Assay cut-off:**

Graph 1. Histogram for determination of the Xpert EV assay cut-off.



The SPC/IC Ct value at which the assay would be called invalid was verified using:

1. Negative assays run during the generation of lot specific parameters
2. Preclinical studies.

Table 6. SPC/IC Ct values verifying “invalid” criteria.

	Valid Ct Range	LSP Negatives	Pre-clinical Negatives
		n = 128	n = 141
SPC/IC	<45		
Mean		34.4	33.5
SD		1.2	1.7
Min		32.2	32.1
Max		40.1	43.9

g. **Cross reactivity/interfering substances**

Studies were conducted with potential interfering substances encountered in CSF. Substances tested were white blood cells, protein, whole blood and hemoglobin.

WBC content was tested using leukocytes (K562 human leukemia cells) spiked into CSF.

To address potential interference from bloody taps, human CSF specimens contaminated with various levels (up to 125,000 RBC/mm³) of blood were tested.

The concentration ranges and the interfering substances found in normal CSF are indicated in Table 7A. Also indicated are the potential ranges found in CSF during

meningitis. Each substance was spiked at levels that could be encountered with normal or meningitis patients.

All tests were performed with CSF spiked with enterovirus serotype CVA9 at 80 TCID₅₀/mL (~3x LOD).

Table 7A. Samples of potentially interfering endogenous substances tested in Xpert EV.

Substance	Concentration range found in normal CSF	Potential CSF concentration range during meningitis	Sample tested with Xpert EV	Concentrations Tested
WBC	0-5 cells/mm ³	5-5000 cells/mm ³	K562 cells	Cells/mm ³ : 0, 3.57, 35.7, 357, 7140
CSF Proteins	13-40 mg/dL	15-217 mg/dL	BSA: IgG (1:1 ratio)	Protein Concentration mg/dL 0, 30, 300, 1,071
Blood	None	N/A	14 Bloody tap Human CSF	0% to approximately 2.5% v/v blood
Hemoglobin	12-18 g/dL RBC	N/A, except in bloody taps	Hemoglobin (Ferrous powder) spiked into CSF	Hemoglobin g/dL 0, 0.36, 0.71, 2.14, 3.6 [Represents approximately v/v blood in CSF, respectively: 0%, 2.5%, 5%, 15%, 25%]

As indicated in Table 7B, positive enterovirus results were obtained even when the highest level of potentially interfering substance was introduced into the assay.

Table 7B. Results of study with potentially interfering endogenous substances tested in Xpert EV.

Interfering substance	Concentration	EV Ct
None (Control n = 8)	N/A	36.1
Protein (n = 4)	1071 mg/dL	38.2
WBC (n = 4)	7,140 cells/mm ³	37.2
Bloody tap, specimen 1	2.5% v/v blood	35.9
Bloody tap, specimen 2	2.5% v/v blood	35.0
Bloody tap, specimen 3	2.5% v/v blood	35.3
Hemoglobin (n = 4)	3.6 g/dL	36.9

2. Comparison studies:

a. *Method comparison with predicate device:* Not applicable

b. *Matrix comparison:* Not applicable

3. Clinical studies:

Performance characteristics of the Xpert EV assay were determined in a multi-site investigational study at six institutions.

To be enrolled in the trial, a patient must have had a lumbar puncture because of meningitis symptoms, and an EV test and/or viral culture test ordered by the physician. The patient must have had sufficient excess CSF volume (greater than or equal to 0.5 ml) and have given written informed consent. Patient samples were excluded if the CSF for nucleic acid testing had been centrifuged or if the Xpert EV assay and assays for the clinical truth determination were not performed within the same freeze-thaw cycle of the specimen. Clinical history of the patients was also considered: clinical signs and symptoms; days since onset of symptoms; maximum temperature; contact history; CSF RBC, WBC, and differential; CSF glucose and total protein; CSF bacterial culture and gram stain; blood glucose; and viral culture from other specimens, if available.

A patient was defined as having EV meningitis (Clinical Diagnosis) if the following criteria were met: clinical evidence consistent with meningitis, laboratory results for CSF Gram stain, CSF bacterial culture, CSF glucose, CSF-blood glucose ratio, CSF total protein concentration, CSF leukocyte count, and either detection of an EV genome in CSF and/or positive CSF EV culture.

Initially 475 patients were submitted for enrollment. Forty-one patients did not meet the study inclusion criteria and were subsequently eliminated from the analysis leaving 434 analyzable subjects of which 255 had results from all the tests described above.

Table 8A. Prospective clinical samples evaluated against “Clinical Diagnosis”.

A total of 199 eligible prospective patients were enrolled, 133 patients had the 6 laboratory results for the “clinical truth” evaluation. The clinical sensitivity and specificity for Xpert EV are shown in the table below.

		Clinical Diagnosis¹	
		+	-
Xpert	+	26	3*
	-	1	103
		27	106

Clinical sensitivity: 96.3% (26/27) 95% CI 81.0-99.9%

Clinical specificity: 97.2% (103/106) 95% CI 91.9-99.4%

Table 8B. Banked prospectively collected clinical samples evaluated against “Clinical Diagnosis”.

A total of 235 eligible retrospective patients were enrolled, 122 patients had the 6 laboratory results for the “clinical truth” evaluation. The clinical sensitivity and specificity for Xpert EV are shown in the table below.

		Clinical Diagnosis¹	
		+	-
Xpert	+	23	3*
	-	0	96
		23	99

Clinical sensitivity: 100% (23/23) 95% CI 85.2-100%

Clinical specificity: 97.0% (96/99) 95% CI 91.4-99.4%

¹A patient was defined as having EV meningitis (Clinical Diagnosis) if the following criteria were met: clinical evidence consistent with meningitis, laboratory results for CSF Gram stain, CSF bacterial culture, CSF glucose, CSF-blood glucose ratio, CSF total protein concentration, CSF leukocyte count, and detection of an EV genome in CSF or positive CSF EV culture

Table 8C. Clinical performance of Xpert EV assay in reference to “Clinical Diagnosis” by age.

The 133 prospective and 122 banked prospectively collected clinical samples were each grouped by age and clinical sensitivity and specificity of each age group are shown in the table below.

Age	Prospective Clinical Samples		Banked Prospectively Collected Clinical Samples	
	Clinical Sensitivity	Clinical Specificity	Clinical Sensitivity	Clinical Specificity
Neonatal (younger than 2 months)	100.0% (14/14)	96.0% (24/25)	100.0% (4/4)	90.0% (18/20)
Pediatrics (2 months to 17 years)	92.3% (12/13)	97.2% (69/71)	100.0% (14/14)	98.1%(51/52)
Adults (18 years and older)	(0/0)	100.0% (10/10)	100.0% (5/5)	100.0% (27/27)
Overall	96.3% (26/27)	97.2% (103/106)	100% (23/23)	97.0% (96/99)

Viral cultures were performed in 73.7% (320/434) of the eligible specimens; the remaining had insufficient CSF for culture. CSF samples from 263 subjects with sufficient excess volume were sent to a designated central laboratory for viral culture. In addition, viral cultures for 114 patient specimens were performed at the enrolling sites. Of these 114 subjects, 57 had viral cultures performed at both the enrolling sites and the central laboratory. Fifty-six of 57 subjects had concordant culture results, one subject had discrepant local and central culture results.

The central laboratory used Super E-Mix Shell Vials for viral culture and the cells were stained with pan enterovirus antibody. The cells that were positive for the pan enterovirus antibody were further stained with indirect immuno-fluorescence antibody for enterovirus identification. Each enrolling site used its own standard procedure for viral culture.

Table 9A. Prospective clinical samples evaluated against viral culture.

Of the 199 eligible prospective specimens 131 had viral culture results. There were no discrepant results relative to enrolling sites and central laboratory viral culture testing. The positive and negative agreements between the Xpert EV and viral culture are shown in the table below.

		Viral Culture	
		+	-
Xpert	+	8	13
	-	0	110
		8	123

Positive agreement: 100.0% (8/8) 95% CI 63.1-100.0%

Negative agreement: 89.4% (110/123) 95% CI 82.6-94.3%

Table 9B. Banked prospectively collected clinical samples evaluated against viral culture.

Of the 235 eligible retrospective specimens 211 had viral culture results. The positive and negative agreements between the Xpert EV and viral culture are shown in the table below.

		Viral Culture	
		+	-
Xpert	+	22	35
	-	1	153
		23	188

Positive agreement: 95.7% (22/23) 95% CI 78.1-99.9%

Negative agreement: 81.4% (153/188) 95% CI 75.1-86.7%

b. Clinical specificity: see above

c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off: NA

5. Expected values/Reference range:

The 434 eligible patients are grouped by age and gender; the number and percentage of positive cases are calculated and shown in the table below.

Table 10: Expected values for Xpert EV in population with signs and symptoms consistent with meningitis.

Age range (years)	Gender	Xpert EV Result		Total
		Positive n (%)	Negative n (%)	
< 1	M	34 (29.3)	82 (70.7)	116
	F	26 (28.3)	66 (71.7)	92
1-5	M	8 (25.0)	24 (75.0)	32
	F	3 (11.1)	24 (88.9)	27
6-10	M	11 (31.4)	24 (68.60)	35
	F	3 (17.6)	14 (82.4)	17
11-15	M	8 (33.3)	16 (66.7)	24
	F	3 (15.0)	17 (85.0)	20
16-21	M	3 (20.0)	12 (80.0)	15
	F	3 (25.0)	9 (75.0)	12
> 21	M	2 (10.0)	18 (90.0)	20
	F	3 (12.5)	21 (87.5)	24
Total		107 (24.7)	327 (75.3)	434

N. Instrument Name: GeneXpert Dx system

O. System Descriptions:

1. Modes of Operation: 4 randomly accessible modules that are each capable of performing separate sample preparation and real-time PCR tests.

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes x or No

3. Specimen Identification: barcodes

4. Specimen Sampling and Handling: automated

GeneXpert Dx System Hardware Components for Automated Sample Processing

Module Hardware Components	Function
Valve Drive	Rotates the cartridge valve body to address the different cartridge chambers.
Syringe Pump Drive	Dispenses fluids to and from the different cartridge chambers.
Ultrasonic Horn	Lyses the bacterial cells and sample prep control.
I-CORE® Module	Performs PCR amplification and detection. As the user inserts the cartridge into the system, the reaction tube component of the cartridge is inserted into the I-CORE module. After sample preparation within the cartridge, the sample and reagent mixture is transferred from the cartridge chamber into the reaction tube. During the amplification process, the I-CORE heater heats up and the fan cools down the reaction tube contents. Two optical blocks positioned within the I-CORE excite the dye molecules that make up the probes and detect the fluorescence emitted. The system uses calibration and data analysis algorithms to determine a relative fluorescence value for each reporter dye after each thermal cycle.

5. Calibration: Optical and thermal calibration of the GeneXpert Dx System is performed by Cepheid at the time of manufacture prior to installation and once yearly or after 1000 runs per module. The user does not calibrate or perform any serviceable functions on the instrument. The normalization function compensates for any optical degradation between calibrations.

The thermal reaction chamber thermistors are calibrated to $\pm 0.50^{\circ}\text{C}$ using National Institute of Standards and Technology (NIST)-traceable standards. During the manufacturing process, the temperature of the heating system is measured at two temperatures: 60°C and 95°C . Calibration coefficients that correct for small errors in the raw thermistor readings of the heaters are stored in the memory of each I-CORE module.

The optical system is calibrated using standard concentrations of individual unquenched fluorescent dye-oligos. For each optical channel, the signal produced by a tube alone (the blank signal) is subtracted from the raw signal produced by the dye-oligo standard to determine the spectral characteristics. Using the individual spectral characteristics of the pure dye-oligos, signals from an unknown mixture of dye-oligos can be resolved into corrected signals for the individual dye-oligos in the mixture.

6. Quality Control:
Before the start of the PCR reaction, the GeneXpert Dx System is programmed to perform a probe check on the EV target and SPC/CIC. The Probe Check control verifies reagent

bead rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. PC is considered to PASS if it meets the predetermined acceptance criteria. If the PC fails in EV target or SPC/CIC; the test will not continue.

External controls must be used for training, proficiency testing and external QC of the GeneXpert Dx System. External controls should be used in accordance with local, state, and federal accrediting organizations as applicable.

External Controls can be prepared by diluting Coxsackievirus A9 strain: Bozek or Coxsackievirus A6 strain C.G. (Gdula) with known negative patient CSF or Synthetic CSF (e.g. SeraCare Life Sciences Inc. Catalog number HSP-515) to approximately 10-1000 TCID₅₀/mL that gives an EV Ct range of 32-35 for the Xpert EV assay.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The “Performance Characteristics” Section above:

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The petition for Evaluation of Automatic Class III Designation for this device is accepted. The device is classified as Class II under regulation 21 CFR 866.3225 with special controls. The special control guidance document "Class II Special Control Guidance Document: Nucleic Acid Amplification Assay for the Detection of Enterovirus RNA" will shortly be available.

S. Other Supportive Device and Instrument Information:

