GeneXpert®
Powered By CEPHEID INNOVATION

# Xpert® EV

REF **GXEV-100N-10**

Cepheid® ***In Vitro*** **Diagnostic Medical Device** CE IVD

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# Xpert® EV

For *In Vitro* Diagnostic Use.

℞ only

## 1 Proprietary Name

Xpert® EV

## 2 Common or Usual Name

Xpert EV Assay

## 3 Intended Use

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 observations 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.**

## 4 Summary and Explanation

The Cepheid® Xpert EV assay is a reverse transcription polymerase chain reaction (RT-PCR) assay used to detect enterovirus RNA in cerebrospinal fluid (CSF) specimens. Enterovirus is taxonomically classified as those viruses consisting of polioviruses, coxsackieviruses, echoviruses, and enteroviruses.[3] Enteroviruses cause a wide range of infections and are most often spread through direct contact with respiratory secretions of an infected person.[1] The common symptoms are fever, severe headache, stiff neck, bright lights hurting the eyes, drowsiness or confusion, and nausea and vomiting. In infants, the symptoms include fever, fretfulness or irritability, difficulty in awakening or loss of appetite.[1] While most infections are either asymptomatic or result in minor febrile illnesses, they often result in hospitalization, especially of infants and children. About 90% of viral meningitis cases are caused by enteroviruses;[2] and enteroviruses are the most common cause of meningitis in the United States, with an estimated 30 000 to 50 000 hospitalizations each year.[3] Enteroviral meningitis usually self-resolves within 7-10 days. However, non-viral causes of meningitis, for example bacterial meningitis, can be serious and may result in disability or death if not treated promptly, therefore meningitis should be taken seriously.[1]

An enterovirus test, together with clinical observation and other clinical information, can help physicians identify patients with enteroviral meningitis to aid in patient management.[4]

## 5 Principle of the Procedure

The GeneXpert Dx System automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples 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 Xpert 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. For a full description of the system, see the *GeneXpert® Dx System Operator Manual.*

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. 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. (Note that in the GeneXpert® Dx System software, CIC is the name for the SPC/IC.) The assay also includes a probe check control to verify reagent rehydration, probe integrity, and reaction-tube filling in the cartridge.

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 (encapsidated RNA pseudovirus), 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.

## 6 Reagents

### 6.1 Materials Provided

The Xpert EV assay kit (GXEV-100N-10) contains sufficient reagents to process 10 samples. The kit contains the following:

| | |
|---|---|
| **Xpert EV Cartridges with Integrated Reaction Tubes** | **10 cartridges/kit** |
| • Bead 1, Bead 2, Bead 3, Bead 4, Bead 5 (freeze-dried) | 1 of each per cartridge |
| **Binding Reagent (Ethanol) (1)** | **10 × 1 mL** |
| **Wash Reagent (2)** | **10 × 3.2 mL** |
| **Elution Reagent (3)** | **10 × 2.0 mL** |
| **Lysis Reagent (Guanidinium Thiocyanate) (4)** | **10 × 300 µL** |
| **CD** | **1 per kit** |
| • Assay Definition File (ADF) | |
| • Instructions to import ADF into GeneXpert software | |
| • Instructions for Use (Package Insert) | |

**Note** Safety Data Sheets (SDS) are available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab.

**Note** The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and post-mortem testing. During processing, there was no mixing of the material with other animal materials.

## 7 Storage and Handling

- Store the Xpert EV cartridges and reagents at 2-28 ºC.
- Do not open a cartridge until you are ready to perform testing.
- Use the cartridge and reagents within 30 minutes after opening the package.
- Do not use cartridges or reagents that have passed the expiration date.
- Do not use any reagents that have become cloudy or discolored.

## 8 Materials Required but Not Provided

- GeneXpert Dx System (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, and Operator Manual
- Printer: If a printer is required, contact Cepheid sales representative to arrange for the purchase of a recommended printer.
- 200-µL pipette
- Sterile 200-µL pipette tips

## 9 Warnings and Precautions

- For *In Vitro* Diagnostic Use Only.
- Do not substitute Xpert EV reagents with other reagents.
- Do not open the Xpert EV cartridge lid except when adding sample and reagents.
- Do not load a Xpert EV cartridge that has been dropped or shaken after you have inserted the sample and reagents.
- Do not load a cartridge that has a damaged reaction tube.
- Do not open used Xpert EV cartridges.
- Do not reuse spent Xpert EV cartridges.
- Do not freeze and thaw the specimens more than two times.
- Do not use specimens that have been centrifuged.
- Lysis Reagent contains guanidine thiocyanate, which can form highly reactive compounds when combined with bleach. If liquid containing this reagent is spilled, clean the area with laboratory detergent and water.
- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. All biological specimens should be handled using standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[5] and the Clinical and Laboratory Standards Institute.[6]
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific national or regional disposal procedures. If national or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.

## 10 Chemical Hazards[7,8]

- UN GHS Hazard Pictogram: 
- Signal Word: DANGER
- **UN GHS Hazard Statements**
  - Highly flammable liquid and vapour
  - Harmful if swallowed
  - Causes skin irritation
  - Causes serious eye irritation
  - Harmful if inhaled
  - May cause drowsiness or dizziness
  - Suspected of causing genetic defects.
  - Toxic to aquatic life
  - Harmful to aquatic life with long lasting effects
- **UN GHS Precautionary Statements**
  - **Prevention**
    - Obtain special instructions before use.
    - Do not handle until all safety precautions have been read and understood.
    - Avoid breathing mists, vapours, and/or spray.
    - Wash thoroughly after handling.
    - Do not eat, drink or smoke when using this product.
    - Use only outdoors or in a well-ventilated area.
    - Avoid release to the environment.
    - Wear protective gloves/protective clothing/eye protection/face protection.

- Use personal protective equipment as required.

- **Response**
  - IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
  - Call a POISON CENTER or doctor/physician if you feel unwell.
  - IF ON SKIN: Wash with plenty of soap and water.
  - Take off contaminated clothing and wash before reuse.
  - Specific treatment, see supplemental first aid information.
  - If skin irritation occurs: Get medical advice/attention.
  - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
  - If eye irritation persists: Get medical advice/attention.
  - IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician if you feel unwell.
  - Rinse mouth.
  - IF exposed or concerned: Get medical advice/attention.
- **Storage/Disposal**
  - Store in a well-ventilated place. Keep container tightly closed.
  - Store locked up.
  - Dispose of content and/or container in accordance with local, regional, national, and/or international regulations.

## 11 Specimen Collection, Transport, and Storage

Collect CSF in a sterile container and transport to the laboratory according to standard operating procedure at your institution. Keep specimens at 2-8 °C until testing or freeze specimens if test will not be performed within 72 hours of collection. Do not freeze and thaw the specimens more than two times. Centrifugation of the specimen is not recommended.

## 12 Procedure

### 12.1 Preparing the Cartridge

To add the sample and reagents into the cartridge (Figure 1):

1. Remove a cartridge and the reagents from the package.
2. Open the Binding Reagent (1) ampule by twisting and breaking off the cap.
3. Insert the tip of the Binding Reagent (1) ampule into cartridge chamber 1 and squeeze the ampule until the entire content is emptied.
4. Open the Wash Reagent (2) ampule by twisting and breaking off the cap.
5. Insert the tip of the Wash Reagent (2) ampule into cartridge chamber 2 and squeeze the ampule until the entire content is emptied.
6. Open the Elution Reagent (3) ampule by twisting and breaking off the cap.
7. Insert the tip of the Elution Reagent (3) ampule into cartridge chamber 3 and squeeze the ampule until the entire content is emptied.
8. Using the 200-µL pipette, add 140 µL of the Lysis Reagent (4) to cartridge chamber 4S. Discard the Lysis Reagent (4) vial.
9. Using the 200-µL pipette, add 140 µL of the sample to cartridge chamber 4S. To prevent large air bubbles from forming, be sure to hold the pipette tip at the top of the chamber and dispense the sample slowly.
10. Close the cartridge lid.

**Important** **Be sure to load the cartridge into the GeneXpert Dx instrument and start the test within 30 minutes of adding the reagents.**

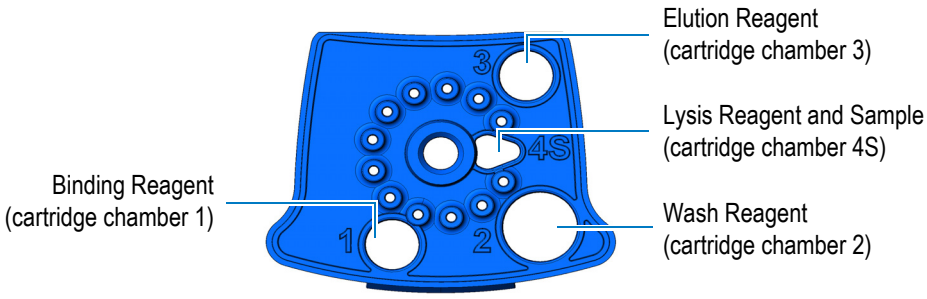


**Figure 1. Xpert EV Cartridge (Top View)**

### 12.2 Starting the Test

**Note** Before you start the test, make sure the Xpert EV assay definition is imported into the software (see the instructions provided with the assay CD). If you do not have the Xpert EV assay CD, contact Cepheid Technical Support.

This section lists the basic steps of running the test. For detailed instructions, see the *GeneXpert Dx System Operator Manual.*

1. Turn on the computer, and then turn on the GeneXpert Dx instrument.
2. On the Windows® desktop, double-click the GeneXpert Dx shortcut icon.
3. Log on to the GeneXpert Dx System software using your user name and password.
4. In the GeneXpert Dx System window, click **Create Test.** The Scan Cartridge Barcode dialog box appears.
5. Scan the bar code on the Xpert EV cartridge. The Create Test window appears. Using the bar code information, the software automatically fills the following boxes: Select Assay, Reagent Lot ID, Cartridge S/N, and Expiration Date.
6. In the Sample ID box, scan or type the sample ID. Make sure you type the correct sample ID. The sample ID is associated with the test results and is shown in the View Results window and all the reports.
7. Click **Start Test.** In the dialog box that appears, type your password.
8. Open the instrument module door with the blinking green light and load the cartridge.
9. Close the module door. Be sure the green light is solid green.
10. When the test is finished, the instrument module light turns off.
11. Wait until the system releases the door lock before opening the module door and removing the cartridge.
12. Follow your laboratory safety guidelines for discarding the cartridge.

## 13 Viewing and Printing Results

For detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual.*

## 14 Quality Control

**CONTROL** Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures.

Each test includes two internal controls to validate the assay: Sample-processing control/internal control and probe check. Test samples are controlled according to the following procedures:

- **Sample-processing control/internal control (SPC/IC)—**The SPC/IC is an encapsidated RNA pseudovirus in the form of a dry bead and is included in each cartridge. The SPC/IC verifies adequate lysis of target EV and sample processing, and detects assay interference.

  It is mixed with the sample to control for adequate sample processing and to monitor the integrity of the RT-PCR assay. The SPC/IC is considered to pass if it meets the validated acceptance criteria. Note that in the GeneXpert Dx System software, CIC is the name for the SPC/IC.

- **Probe check—**Before the start of the PCR reaction, the system performs a probe check on both the EV target and the SPC/IC to verify reagent bead rehydration and reaction-tube filling. Each probe check is considered to pass if it meets the validated acceptance criteria.

- **External Controls—**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 $TCID_{50}$/mL that gives an EV $C_t$ range of 32 - 35 for the Xpert EV assay.

## 15 Interpretation of Results

The results are available in the GeneXpert Dx System View Results window. Possible results are described in this section.

**Note** In the GeneXpert Dx System View Results window, the SPC/IC is displayed as CIC in the Analyte Name column.

**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.**

**Table 1. Xpert EV Results and Interpretation**

| Result | Interpretation |
|---|---|
| **POSITIVE**<br><br>**Figure 2** | EV target nucleic acid is detected (GeneXpert Dx System—**View Results** window. Note that the SPC/IC is displayed as CIC.):<br>• EV—POS<br>• CIC (SPC/IC)—NA (When EV titer is high, the RT-PCR for the SPC might be suppressed.)<br>• Probe Check—PASS<br>• **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.** |
| **NEGATIVE**<br><br>**Figure 3** | EV target nucleic acid is not detected, but SPC meets acceptance criteria (GeneXpert Dx System—**View Results** window. Note that the SPC/IC is displayed as CIC.):<br>• EV—NEG<br>• CIC (SPC/IC)—PASS<br>• Probe Check—PASS<br>• **Negative Xpert EV results do not rule out enterovirus as causes of meningitis but that enterovirus was not detected.** |

**Table 1. Xpert EV Results and Interpretation (Continued)**

| Result | Interpretation |
|---|---|
| **INVALID**<br><br>**Figure 4** | The presence or absence of EV target nucleic acid cannot be determined, repeat test with extra specimen. SPC/IC does not meet acceptance criteria, the sample was not properly processed, or PCR is inhibited (GeneXpert Dx System—View Results window. Note that the SPC/IC is displayed as CIC.):<br>• EV—INVALID<br>• CIC (SPC/IC)—FAIL<br>• Probe Check—PASS |
| **ERROR** | The presence or absence of EV target nucleic acid cannot be determined, repeat test with extra specimen. The Probe Check control failed probably due to the reaction tube being filled improperly, a probe integrity problem was detected, or the assay aborted:<br>• EV—NO RESULT<br>• CIC (SPC/IC)—NO RESULT<br>• Probe Check—FAIL |
| **NO RESULT** | The presence or absence of EV target nucleic acid cannot be determined, repeat test with extra specimen. Insufficient data were collected to produce a test result (for example, the operator stopped a test that was in progress):<br>• EV—NO RESULT<br>• CIC (SPC/IC)—NO RESULT<br>• Probe Check—NA |

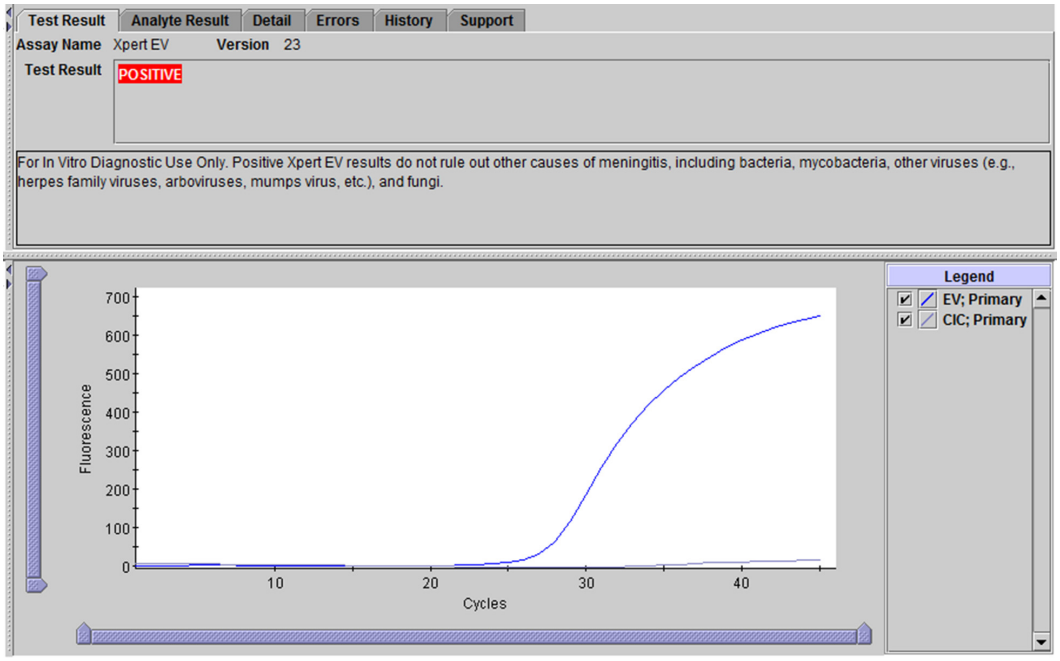


**Figure 2. Xpert EV Positive Result**
**(GeneXpert® Dx System—View Results Window. Note that the SPC/IC is displayed as CIC.)**

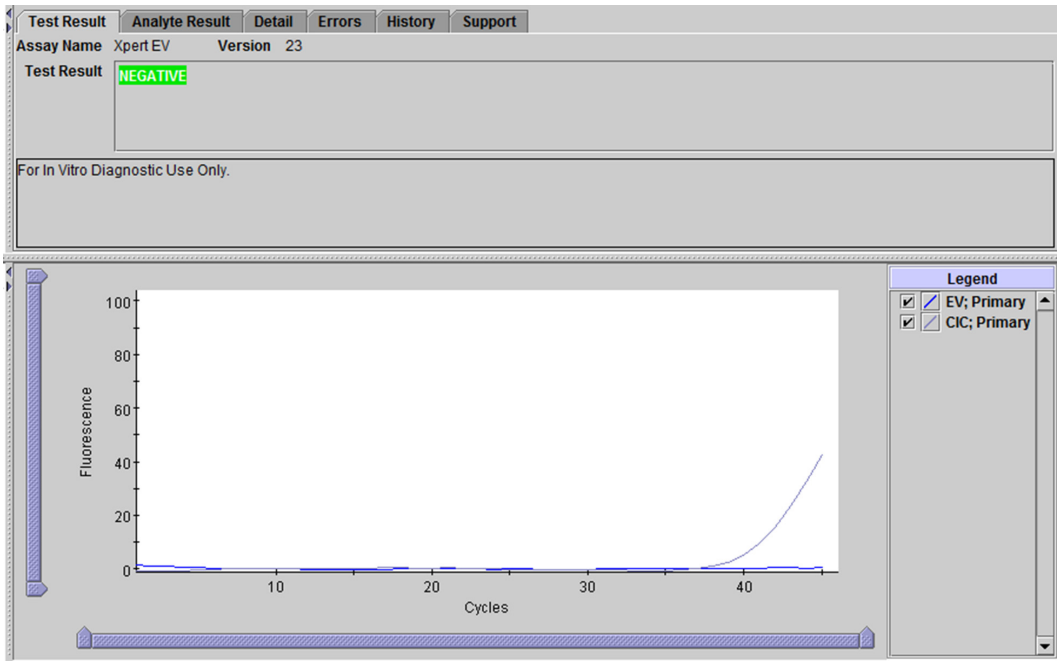


**Figure 3. Negative Result**
**(GeneXpert® Dx System—View Results Window. Note that the SPC/IC is displayed as CIC.)**

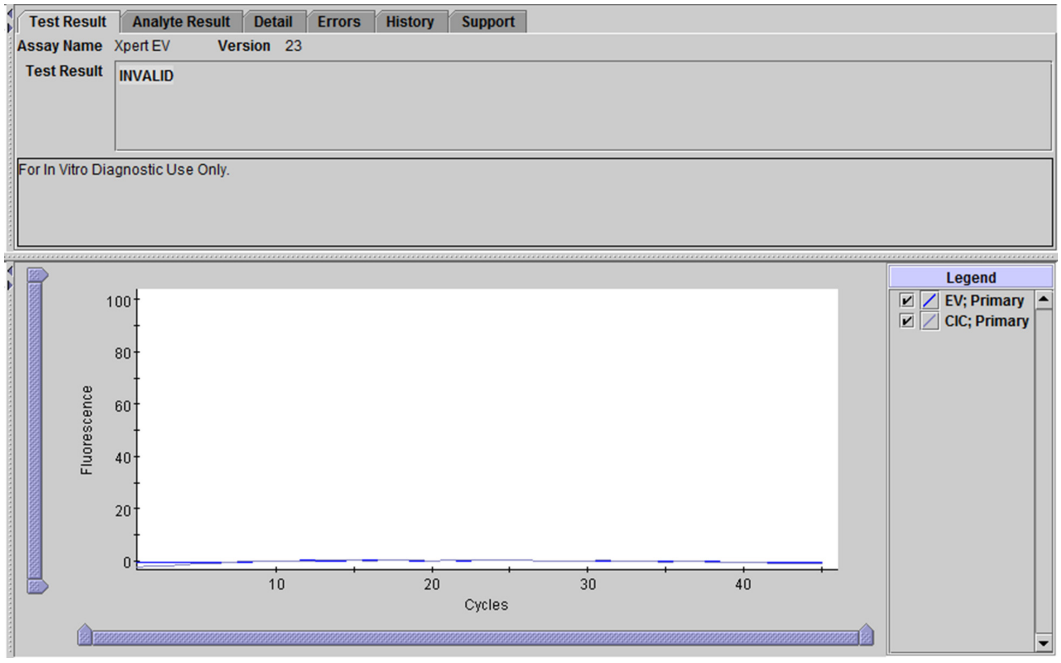


**Figure 4. Xpert EV Invalid Result**
**(GeneXpert® Dx System—View Results Window. Note that the SPC/IC is displayed as CIC.)**

# 16 Reasons to Repeat the Assay

## 16.1 Reasons to Repeat the Test

Repeat the assay with fresh sample if the following results are generated:

- An **INVALID** result indicates that the controls SPC/IC failed. The sample was not properly processed or PCR is inhibited.
- An **ERROR** result indicates that the Probe Check control failed and the assay was aborted possibly due to the reaction tube being filled improperly, a reagent probe integrity problem was detected, or because the maximum pressure limits were exceeded.
- A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

# 17 Limitations

- Results from the Xpert EV assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician. An Xpert EV assay positive result does not rule out the presence of another pathogen like bacteria in CSF. As with any molecular assay, false positive results are always a possibility. Rare occurrences of simultaneous mixed bacterial-viral meningitis have been reported in the literature.[9, 10, 11] The performance of the Xpert EV assay was validated using the procedures provided in this package insert and with the Cepheid GeneXpert Dx System only. Modifications should not be made to these procedures as they might alter the performance of the test.
- The Xpert EV assay is for the detection of enterovirus only. Negative test results do not rule out the presence of enterovirus. This test does not rule out the possibility of Herpes-induced meningitis or fungal meningitis; additional testing is required to rule out these infections.
- Erroneous test results might occur from improper specimen collection; failure to follow the recommended sample collection, handling, and storage procedures; technical error; sample mix-up; or because the number of organisms in the specimen is too low to be detected by the test. Careful compliance with the instructions in this insert is necessary to avoid erroneous results.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown variants and may result in a false negative result.

**Caution**

**As with other diagnostic procedures, 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.**

# 18 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/$mm^3$) of blood were tested.

The concentration ranges and the interfering substances found in normal CSF are indicated in Table 2. 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 $TCID_{50}$/mL (~3x LOD).

**Table 2. 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/$mm^3$:<br>0, 3.57, 35.7, 357, 7140 |
| CSF Proteins | 13-40 mg/dL | 15-217 mg/dL | BSA: IgG<br>(1:1 ratio) | Protein Concentration mg/dL<br>0, 30, 300, 1,071 |

**Table 2. Samples of Potentially Interfering Endogenous Substances Tested in Xpert EV (Continued)**

| Substance | Concentration range found in normal CSF | Potential CSF concentration range (during meningitis) | Sample tested with Xpert EV | Concentrations Tested |
|---|---|---|---|---|
| Blood | None | Not applicable | 14 bloody tap Human CSF | 0% to approximately 2.5% v/v blood |
| Hemoglobin | 12-18 g/dL RBC | Not applicable except in bloody taps | Hemoglobin (Ferrous powder) spiked into CSF | HgB g/dL<br>0, 0.36, 0.71, 2.14, 3.6<br>[Represents approximately v/v blood in CSF, respectively:<br>0%, 2.5%, 5%, 15%, 25%] |

As indicated in Table 3, positive enterovirus results were obtained even when the highest level of potentially interfering substance was introduced into the assay.

**Table 3. Results of Study with Potentially Interfering Endogenous Substances Tested in Xpert EV**

| Interfering substance | Concentration | EV $C_t$ |
|---|---|---|
| None (Control n = 8) | Not applicable | 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 |

# 19 Performance Characteristics

## 19.1 Clinical Performance

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.

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 Table 4.

**Table 4. Prospective Clinical Samples Evaluated Against "Clinical Diagnosis"**

| Clinical Diagnosis[a] | | | |
|---|---|---|---|
| Xpert EV | | + | - |
| | + | 26 | 3 |
| | - | 1 | 103 |
| Totals | | 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%

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 Table 5.

**Table 5. Banked prospectively collected clinical samples evaluated against "Clinical Diagnosis"**

| Clinical Diagnosis[a] | | | |
|---|---|---|---|
| Xpert EV | | + | - |
| | + | 23 | 3 |
| | - | 0 | 96 |
| Totals | | 23 | 99 |

Clinical Sensitivity: 100% (23/23); 95% CI 85.2 - 100%
Clinical Specificity: 97.0% (96/99); 95% CI 91.4 - 99.4%

a. 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 detection of an EV genome in CSF or positive CSF EV culture.

The 133 prospective and 122 banked prospectively collected clinical samples were each grouped by age. Clinical sensitivity and specificity of each age group are shown in Table 6.

**Table 6. Clinical Performance of Xpert EV Assay in Reference to "Clinical Diagnosis" by Age**

| 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.

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 Table 7.

**Table 7. Prospective Clinical Samples Evaluated Against Viral Culture**

| | | Viral Culture | |
|---|---|---|---|
| | | + | - |
| **Xpert EV** | + | 8 | 13 |
| | - | 0 | 110 |
| Totals | | 8 | 123 |

Positive agreement: 100.0% (8/8) 95% CI 63.1-100.0%
Negative agreement: 89.4% (110/123) CI 82.65-94.3%

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 Table 8.

**Table 8. Banked Prospectively Collected Clinical Samples Evaluated Against Viral Culture**

| | Viral Culture | | |
|---|---|---|---|
| | | + | - |
| **Xpert EV** | + | 22 | 35 |
| | - | 1 | 153 |
| Totals | | 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%

The 434 eligible patients are grouped by age and gender; the number and percentage of positive cases are calculated and shown in Table 9.

**Table 9. 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 | 3 (31.4) | 24 (68.6) | 35 |
| | F | 3 (17.6) | 14 (82.4) | 17 |

**Table 9. Expected Values for Xpert EV in Population with Signs and Symptoms Consistent with Meningitis (Continued)**

| Age range (years) | Gender | Xpert EV Result | | Total |
|---|---|---|---|---|
| | | Positive n (%) | Negative n (%) | |
| 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 |

### 19.2 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. The estimated analytical sensitivity is shown in Table 10 below.

Sixty of the serotypes were tested and the estimated $TCID_{50}$/mL that these serotypes can be detected are shown in Table 10.

**Table 10. 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 |

**Table 10. Estimated Analytical Sensitivity (Continued)**

| Species | Serotype | Estimated $TCID_{50}$/mL |
|---|---|---|
| B | Echo 8 | 0.10 |
| B | Echo 9 | 2.00 |
| B | Echo 11 | 40.00 |
| B | Echo 12 | 1.58 |
| B | Echo 13 | 0.01 |
| 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 | Coxsackie A11 | 0.11 |
| C | Coxsackie A13 | 13.27 |
| C | Coxsackie A15 | 0.0032 |
| 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 [a] | 2.00 |
| Poliovirus | Poliovirus 2 [a] | 0.40 |
| Poliovirus | Poliovirus 3 [a] | 20.00 |

a. WARNING: When working with Polioviruses, ensure that appropriate biosafety-level containment procedures are followed.

## 20 Analytical Specificity

The primer and probe sequences used in the Xpert EV assay do not detect nucleic acid extracted from the following organisms known to cause meningitis-like symptoms: EBV, HSV-1, HSV-2, HHV-6, HHV-7, AdV-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 meningitides, Citrobacter freundii,* and *Citrobacter koseri,* nor did the Xpert EV assay generate any detectable amplicons when "whole organisms" of the listed pathogens were processed through the Xpert EV cartridge. The table below presents the organisms tested and the concentration for each organism tested.

Whole organisms were tested for specificity in the Xpert EV assay and the concentrations of the organisms tested are shown in Table 11.

**Table 11. Analytical Specificity for the Xpert EV Assay**

| Organism | No. organisms/test |
|---|---|
| HHV-6 | $3.1 \times 10^6$ particles |
| HHV-7 | $1.4 \times 10^7$ particles |
| CMV | 700 $TCID_{50}$ |
| EBV | 140 $TCID_{50}$ |
| HSV-1 | $1.4 \times 10^5$ $TCID_{50}$ |
| HSV-2 | $1.4 \times 10^5$ $TCID_{50}$ |
| AdV-2 | $1.4 \times 10^{12}$ $TCID_{50}$ |
| Measles | 700 $TCID_{50}$ |
| Mumps | $1.4 \times 10^4$ $TCID_{50}$ |
| Parainfluenza 1 | $1.4 \times 10^3$ $TCID_{50}$ |
| Parainfluenza 2 | $7 \times 10^3$ $TCID_{50}$ |
| Parainfluenza 3 | $1.4 \times 10^4$ $TCID_{50}$ |
| Influenza A | $3.5 \times 10^4$ $TCID_{50}$ |
| Influenza B | $3.5 \times 10^4$ $TCID_{50}$ |
| VZV | 14 $TCID_{50}$ |
| Group B Streptococcus | $7 \times 10^6$ cells |
| H. influenzae B | $7 \times 10^6$ cells |
| H. influenzae non-B | $7 \times 10^5$ cells |
| E.coli | $7 \times 10^6$ cells |
| N. meningitides | $7 \times 10^6$ cells |
| C. freundii | $7 \times 10^6$ cells |
| C. koseri | $7 \times 10^6$ cells |

## 21 Analytical Sensitivity

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).

The LOD of the five (5) serotypes, one from each of the enterovirus species are shown in Table 12.

**Table 12. Limit of Detection for Five (5) Serotypes**

| Serotype | Limit of Detection ($TCID_{50}$/mL) |
|---|---|
| CVA9 | 80.0 |
| EV70 | 1.3 |
| PV1 | 4.0 |
| CVA17 | 1.0 |
| CVA6 | 33.0 |

## 22 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 near the limit of detection.

The percent of agreement, the average Ct values for each concentration, the associated standard deviations, percent coefficient of variation for between day and between site for the multi-center reproducibility study are shown in Table 13.

**Table 13. Summary of the 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[a] | 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[a] | 33.8 | 0 | 0.00% | 0 | 0.00% | 0.414 | 1.22% |
| Total Agreement | 120/120 | 120/120 | 118/118 | | | | | | | |
| % Agreement | 100.00% | 100.00% | 100.00% | | | | | | | |

a. Two samples did not give any GeneXpert result.

In order to further stress the system, a second study was performed. An internal reproducibility study was conducted over four different days on multiple GeneXpert instruments (31) and ICORE modules (121). 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. The negative sample was tested 20 times whereas two positive samples at the two concentrations were tested five (5) times per day. 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 classified.

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 Table 14.

**Table 14. Summary of the Second Reproducibility Study Results**

| Specimen ID | | Total Agreement—$C_t$ 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% | |
| Number of instruments used | | 10 | 11 | 10 | 10 | 31 | |
| Number 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

## 23 References


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## 25 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag number


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Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en/CustomerSupport.

## 26 Table of Symbols

| Symbol | Meaning |
|---|---|
|  | Catalog number |
|  | *In vitro* diagnostic medical device |
|  | Do not reuse |
|  | Batch code |
|  | Consult instructions for use |
|  | Caution |
|  | Manufacturer |
|  | Country of manufacture |
|  | Contains sufficient for <n> tests |
|  | Control |
|  | Expiration date |
|  | CE marking – European Conformity |
|  | Temperature limitation |
|  | Biological risks |
|  | For prescription use only |
|  | Flammable liquids hazard |
|  | Warning |
|  | Authorized Representative in the European Community |
|  | Authorized Representative in Switzerland |
|  | Importer |
|  | Aspiration hazard |

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