Assay Training: Xpert® EV

For CE-IVD and US-IVD Use Only

CE IVD In Vitro Diagnostic Medical Device

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Training Agenda

- Xpert EV Training
  - Reagents
  - Sample collection
  - Kit storage and handling
  - Preparing the cartridge
  - Quality control
  - Results analysis
- Discussion

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Xpert EV Training Objectives

At the end of the training, users will be able to:

- Store and handle the Xpert EV cartridge kit.
- Follow proper laboratory safety precautions.
- Identify appropriate specimen types and transport specimens.
- Prepare a cartridge and run the assay.
- Report and understand the various software generated results.
- Understand the assay control strategy.

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Xpert® EV

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The Cepheid Solution

- Detection of enterovirus (EV) RNA in cerebrospinal fluid (CSF) specimens
- On-board internal controls for each sample
  - Probe Check Control (PCC)
  - Sample Processing Control (SPC)
- Closed cartridge system minimizes risk of contamination
- On-demand results
- Random access

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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.

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System and Reagent Requirements

GeneXpert Systems

- GeneXpert Software v2.1 or higher

Test Kits (US-IVD and CE-IVD)

- GXEV-100N-10

Materials Required but not Provided

- 200-µL pipette
- Sterile 200-µL filter barrier pipette tips
- Personal Protective Equipment (PPE)
- 1:10 dilution of bleach
- 70% ethanol or denatured ethanol

Optional

- Uninterruptible Power Supply/ Surge Protector
- Printer

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Good Laboratory Practice

Personnel Protective Equipment (PPE)	• Wear clean lab coats and gloves • Change gloves between processing samples
Lab Bench area	• Clean work surfaces routinely with: ✓ 1:10 dilution of household bleach ✓ 70% Ethanol Solution • After cleaning, ensure work surfaces are dry
Specimens, Samples, and Kits Storage	• Store specimens and sample away from kit to prevent contamination
Equipment(s)	• Use filtered tips when recommended • Follow the manufacturer's requirements for calibration and maintenance of equipment(s)

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Kit Handling

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Xpert EV Kit Contents

Xpert EV	
Catalog Number	GXEV-100N-10
Cartridges per Kit	10
Reagent Vials	Binding Reagent
	Wash Reagent
	Elution Reagent
	Lysis Reagent
Kit CD	Assay Definition File (ADF)
	Assay Import Instructions
	Package Insert (PDF)
Storage	2- 28 ˚C

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Xpert EV Kit Storage and Handling

- Store the Xpert EV cartridges and reagents at 2–28°C
- Follow your institution's safety procedures for working with chemicals and handling biological samples
- Open the cartridge lid only when adding the sample, close the lid, and proceed with processing

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Warnings and Precautions

- Do not shake the cartridge
- Do not use a cartridge… :
  - if it appears wet, has leaked, or if the lid seal appears to have been broken
  - if it appears damaged
  - that has been dropped after removing it from packaging
  - that has been dropped or shaken after you have added the sample
  - that has a damaged reaction tube
  - that has been used; each cartridge is single-use to process one test
  - is expired
- Do not reuse pipettes

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Waste Disposal

- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents and require use of 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.

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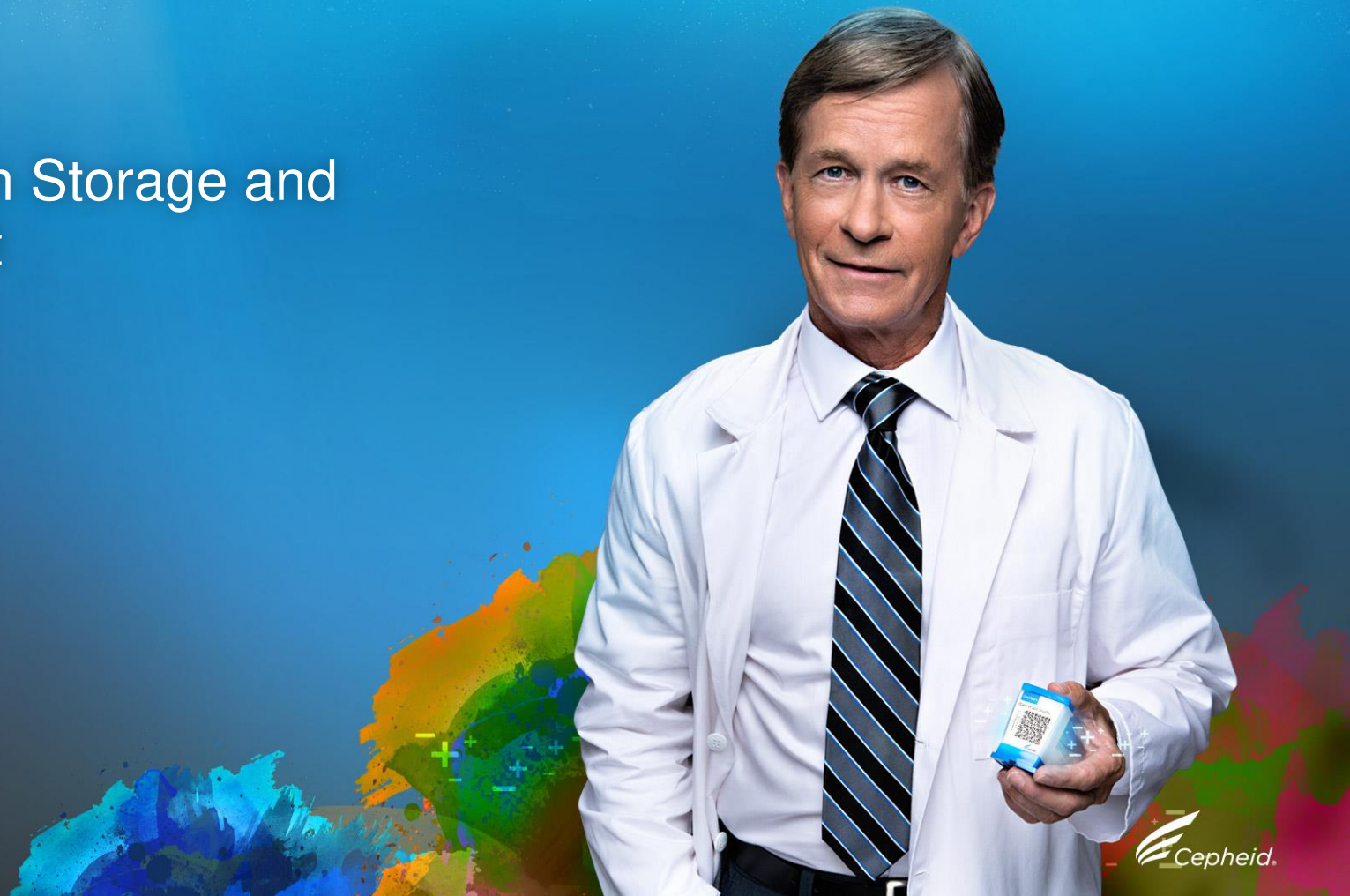
Specimen Storage and Transport



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Specimen Handling

Specimen	Storage
Cerebrospinal fluid (CSF) in sterile container	2-8 ºC for up to 72 hours following specimen collection
	The specimen may also be frozen at -20 or -80 ºC if test will not be performed within 72 hours *Do not freeze and thaw specimen more than twice

⚠ Centrifugation of the specimen is not recommended.

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Xpert EV Testing Protocol

Xpert EV Cartridge Preparation

Refer to the package insert for detailed instructions, precautions, and warnings.

For a copy of the MSDS, visit www.cepheid.com or www.cepheidinternational.com

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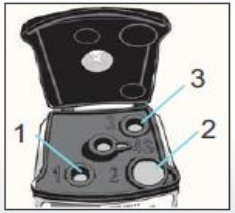
A better way.

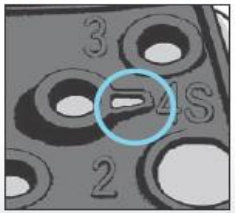
1 Obtain one cartridge, three ampoules, and one vial for each sample.

2 Open the cartridge lid.

3 Open, and then add ampoule 1 into 1, ampoule 2 into 2, and ampoule 3 into 3.



4 Pipette 140 µL of the Lysis Reagent into chamber 4S, and then pipette 140 µL of the sample into chamber 4S.



5 Close the cartridge lid.

6 Start the test within the timeframe specified in the package insert.

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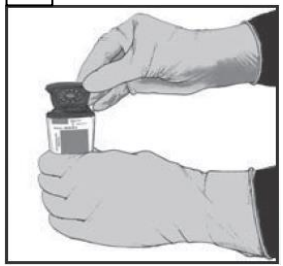
EV Cartridge Preparation

1

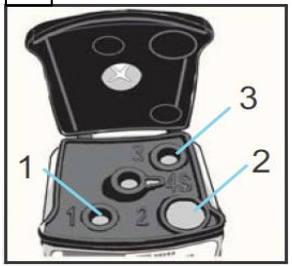
Obtain one cartridge, three ampoules, and one vial for each sample.

2



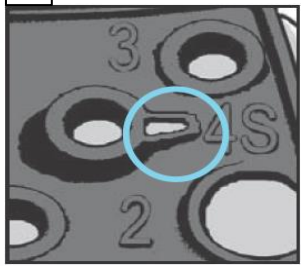
Open the cartridge lid.

3



Open, and then add ampoule 1 into 1, ampoule 2 into 2, and ampoule 3 into 3.

4



Pipette 140 µL of the Lysis Reagent into chamber 4S, and then pipette 140 µL of the sample into chamber 4S.

5

Close the cartridge lid.

6

Start the test within the timeframe specified in the package insert.

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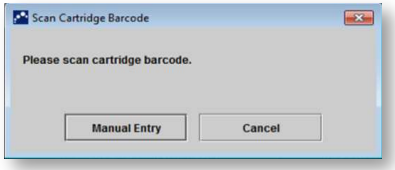
Run a Test

1 Create Test

GeneXpert

Start the test within 30 minutes after adding the sample to the cartridge

2 Scan barcodes: Cartridge/ Patient and/or Sample ID

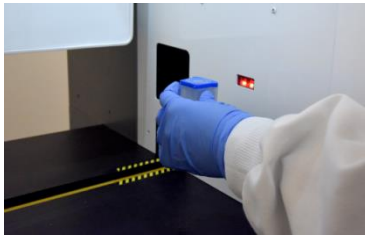


By default, do not click on **Manual Entry** or **Cancel**

3 Scan the cartridge

GeneXpert Infinity

Place the cartridge on the conveyor within 30 minutes of adding the sample.



"For complete details on how to run a test, refer to the Package Insert and the GeneXpert Dx or Xpertise Operator Manuals.

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Create a Test on GeneXpert Dx Software

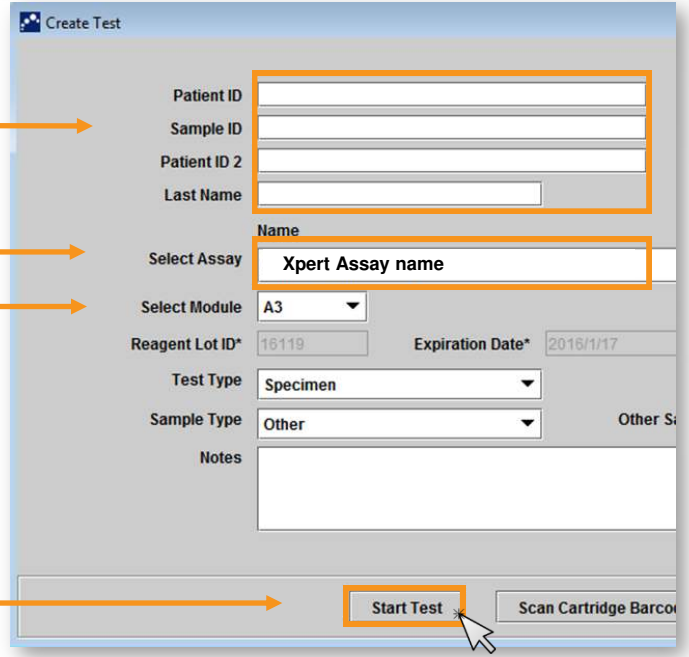
4 Complete the fields as required

5 The Assay Protocol is selected automatically

6 The module is selected automatically

7 Click on Start Test

8 A green light will flash on the module
Load the cartridge into module and close the door



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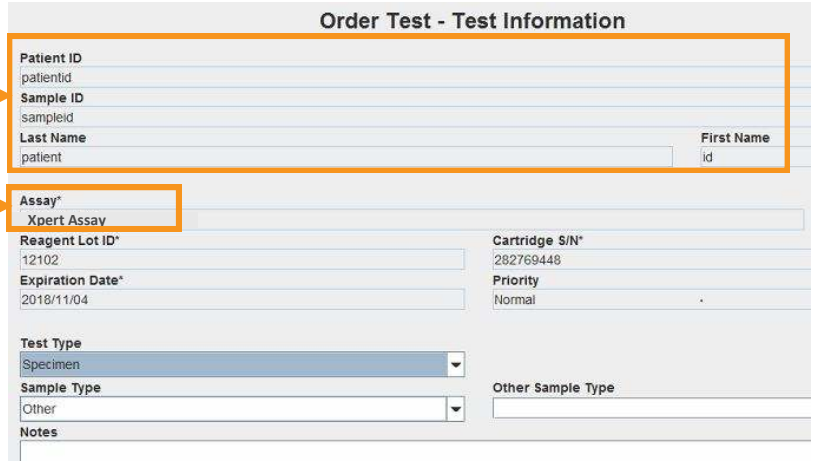
Create a Test on Xpertise Software

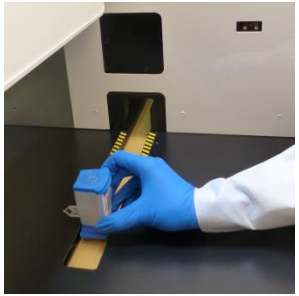
4 Complete the fields as required

5 The Assay Name Protocol is selected automatically

6 Click on SUBMIT



7 Place the cartridge into the conveyor belt



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Automated Xpert Protocol

1. Add the sample to the cartridge
2. Place the cartridge into the instrument.
3. Nucleic acids are purified.
4. Purified nucleic acids mix with PCR reagents.
5. Simultaneous amplification and detection occurs.
6. Results are ready to view.

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Quality Controls

Refer to the Package Insert for complete details

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Cepheid Assay Control Strategy

CONTROL

- Each Xpert cartridge is a self-contained test device.
- Cepheid designed specific molecular methods to include internal controls that enable the system to detect specific failure modes within each cartridge.
  - Probe Check Control (PCC)
  - Sample Processing Control (SPC)

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Internal Quality Controls

CONTROL

- **Probe Check Controls (PCC)**
  - Before the PCR step, fluorescence signal is measured on all probes and compared with default factory settings to monitor
    - bead rehydration
    - reaction tube filling
    - probe integrity
    - dye stability

- **Sample Processing Controls (SPC)-** displayed as CIC
  - Verifies adequate sample processing
  - Verifies lysis, presence of the organism and detects PCR inhibition
  - Should be positive in a negative sample
  - Can be positive or negative in a positive sample

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Results Analysis

Refer to the Package Insert for complete details

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Results Summary

Result displayed	EV	CIC
POSITIVE	+	+/-
NEGATIVE	-	+
INVALID	-	-
ERROR	NO RESULT	NO RESULT
NO RESULT	NO RESULT	NO RESULT

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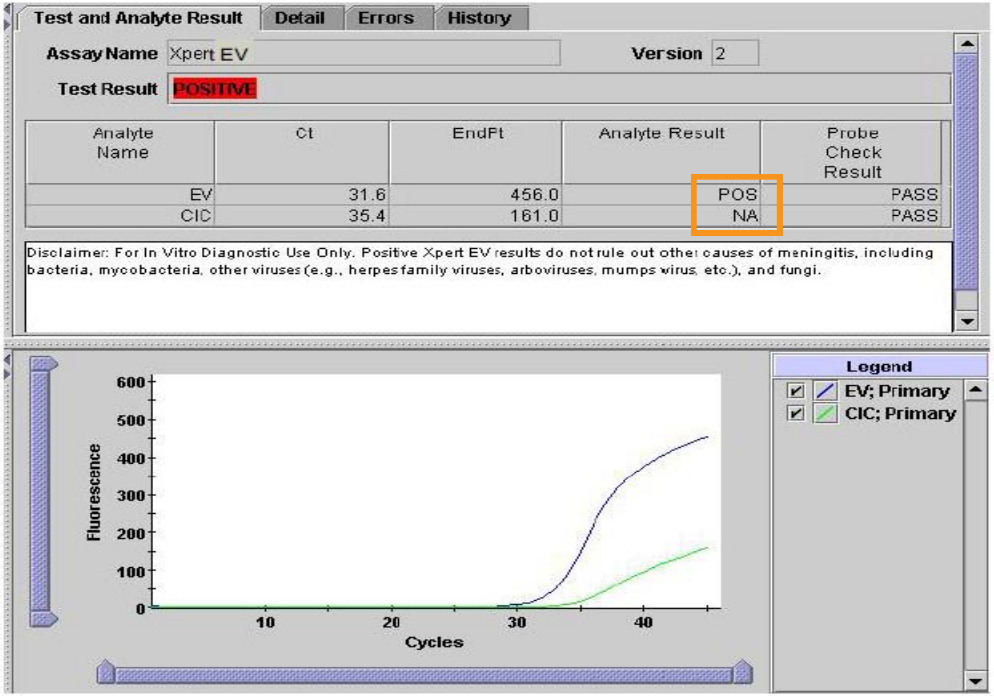
EV Positive

Test Result POSITIVE

EV target nucleic acid is detected.

- CIC (SPC/IC)—NA
- Probe Check—PASS
  All probe check results pass.

Positive Xpert EV results do not rule out other causes of meningitis, including bacteria, mycobacteria, other viruses, and fungi.



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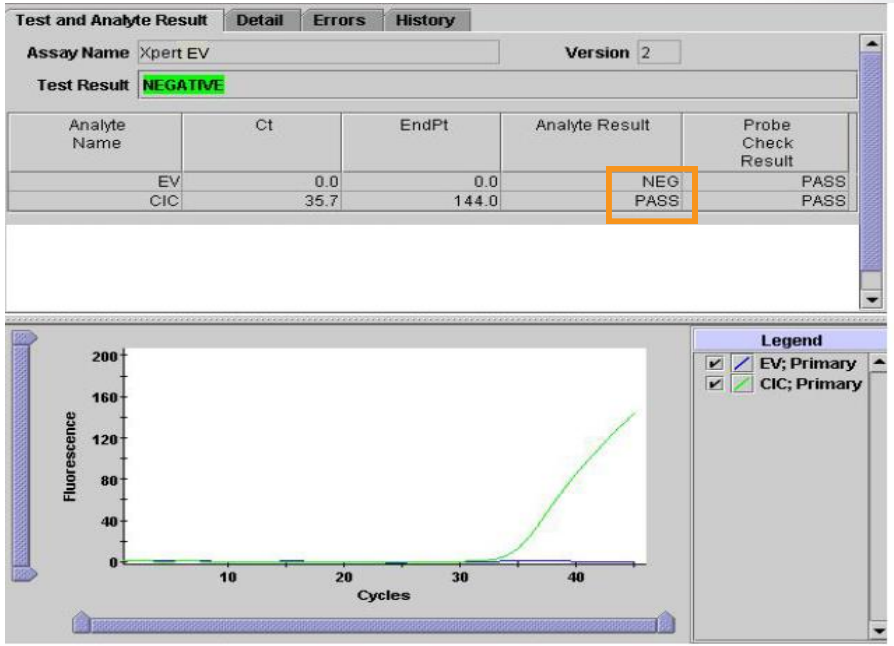
EV Negative

EV target nucleic acid is not detected.
EV—NEG

- CIC (SPC/IC) – PASS; SPC has a Ct within the valid range and endpoint above the minimum setting.
- Probe Check – PASS; all probe check results pass.

Negative Xpert EV results do not rule out enterovirus as the cause of meningitis, but indicate that enterovirus was not detected in the specimen.



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Troubleshooting

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Factors That Negatively Affect Results

- Improper specimen collection
  - The viral load in the specimen is below the detection limit of the test
  - Performance with other specimen types has not been assessed
- Improper transport or storage of collected specimen
  - Storage and transport conditions are specimen specific
  - Refer to the Package Insert for the appropriate handling instructions
- Improper testing procedure
  - Modification to the testing procedures may alter the performance of the test
  - Careful compliance with the package insert is necessary to avoid erroneous results

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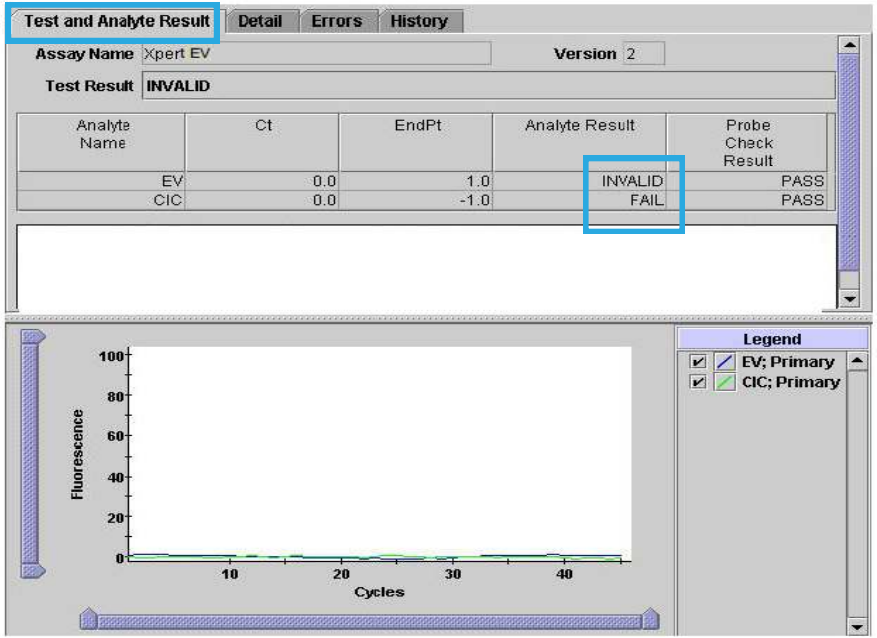
INVALID

Presence or absence of EV cannot be determined.

SPC/IC does not meet the acceptance criteria, the sample was not properly processed, or PCR was inhibited.

- EV—INVALID
- CIC (SPC/IC)—FAIL
- Probe Check—PASS



Repeat test according to the instructions in the Retest Procedure within the Package Insert.

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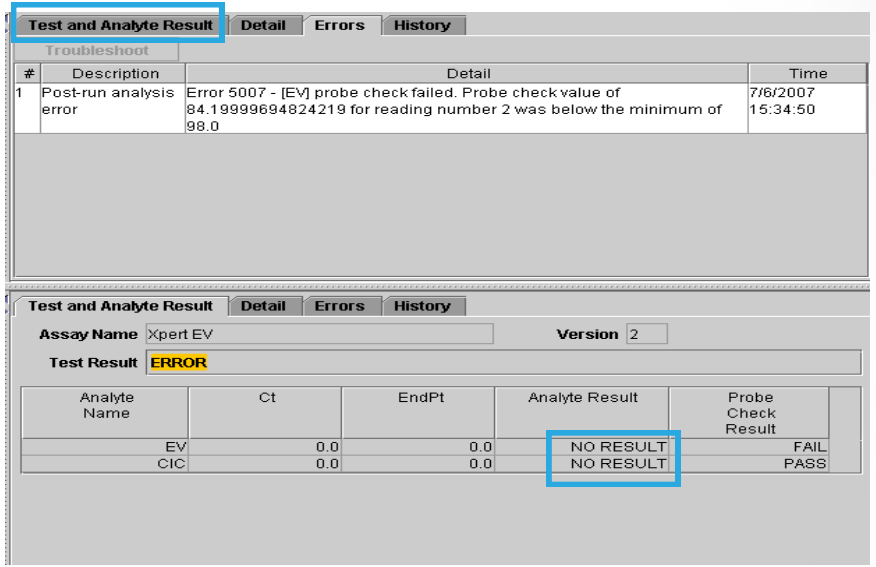
ERROR

Test Result ERROR

Presence or absence of EV cannot be determined.

- EV—NO RESULT
- CIC (SPC/IC)—NO RESULT
- Probe Check—FAIL

Repeat test according to the instructions in the Retest Procedure within the Package Insert.



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No Result

The presence or absence of EV cannot be determined.

- EV—NO RESULT
- CIC (SPC/IC)—NO RESULT
- Probe Check—NA

Possible Causes

A NO RESULT indicates that insufficient data were collected.

- Test was stopped with stop test button
- Electrical failure

Solution

- Secure the power
- Repeat the test with a new cartridge

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EV Retest Procedure

1

Discard used cartridge.

Follow your institution's safety guidelines for disposal of cartridges.

2

Obtain a fresh sample.

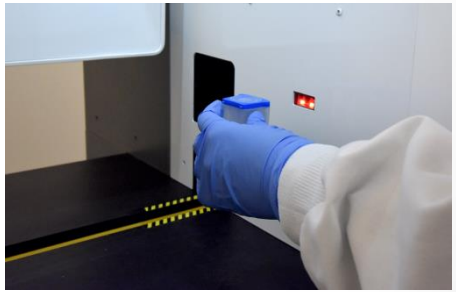
3

Obtain a new cartridge.

Process the sample per the package insert.

4

Run the test on the system.



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Interfering Substances

Positive enterovirus results were obtained even when the highest level of potentially interfering substance was introduced into the assay.

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

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Technical Assistance

- Before contacting Cepheid Technical Support, collect the following information:
  - Product name
  - Lot number
  - Serial number of the System
  - Error messages (if any)
  - Software version and, if applicable, Computer Service Tag number

- Log your complaint online using the following link ***http://www.cepheid.com/us/support*** **:** *Create a Support Case*

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US	+ 1 888 838 3222	techsupport@cepheid.com
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Brazil and Latin America	+ 55 11 3524 8373	latamsupport@cepheid.com
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