Assay Training : Xpert® Norovirus

Cepheid Technical Support
CE-IVD

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Training Agenda

- **Xpert Norovirus Training**
  - Reagents
  - Sample collection
  - Kit storage and handling
  - Precautions
  - Preparing cartridge
- **Quality Control**
- **Results analysis**
- **Discussion and Q&A**

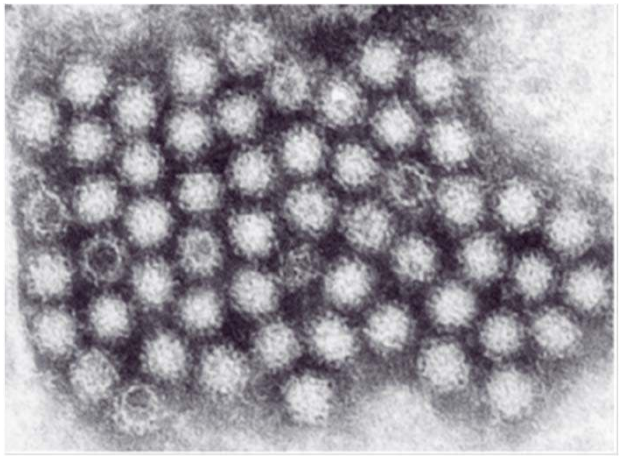
Xpert Norovirus Training Objectives

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

- Properly store and handle the Xpert Norovirus cartridge and assay kit.
- Follow proper laboratory safety precautions.
- Collect appropriate specimen and transport specimen.
- Perform the cartridge set up and run the assay.
- Report the various software generated-results.
- Understand assay control strategy.

Introduction on Disease State

What is Norovirus?



- Norovirus is the most common cause of infectious gastroenteritis (diarrhea and vomiting) and accounts for 50% of viral gastroenteritis around the world.[1]
- This is a highly contagious RNA virus that can be transmitted by fecal-contaminated food or water, person-to-person contact, or aerosolization.
- Immunocompromised patients are at greatest risk including elderly and children.
- Norovirus outbreaks in healthcare institutions require immediate implementation of infection control measures and ward closures.
- Patients suspected of infection could be admitted into isolation pending the result.
- Norovirus Genogroup I (GI) and Norovirus Genogroup II (GII) are responsible for the majority of cases in humans.

1. CDC MMWR 2011 60 (3)

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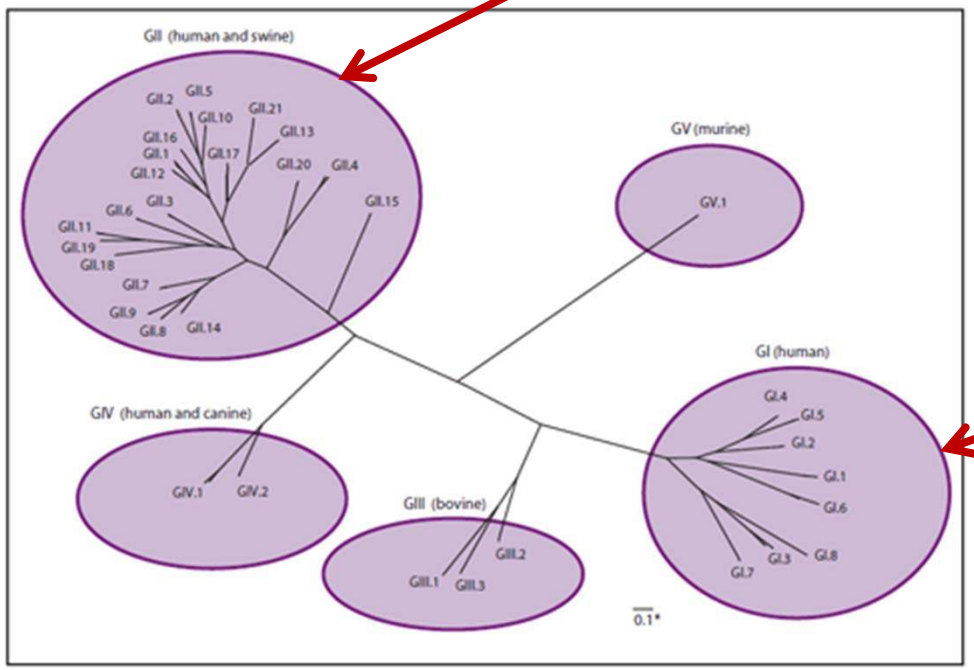
Classification of Noroviruses

Noroviruses are classified into 5 genogroups (I – V) and 32 genotypes, based on sequence diversity in the complete capsid protein (VP1).

GII



→ Group I, II and IV are human pathogens.

GI

GII is the most dominant genotype (70-80%) worldwide.

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The Cepheid Solution

- **Simultaneous detection**
  - **GI and GII**
- **Two controls for each individual sample**
  - **Sample Processing Control (SPC)**
  - **Probe Check control**
- **High sensitivity and specificity**
- **Simple and Easy to Use**
  - **Closed cartridge system**
- **Results in approximately 90 minutes**
  - **EAT (Early Assay Termination)**
- **On-demand results 24/7**
- **Random access**

Intended Use

The Xpert Norovirus Assay, performed on the Cepheid GeneXpert® Instrument Systems, is a qualitative *in vitro* diagnostic test for the rapid identification and differentiation of norovirus genogroup I and genogroup II from raw or unpreserved unformed stool specimens collected from individuals with symptoms of acute gastroenteritis. The test utilizes automated real-time reverse transcriptase polymerase chain reaction (rRT-PCR) to detect norovirus RNA. The Xpert Norovirus Assay is intended to aid in the diagnosis of norovirus infections.

System and Reagent Requirements

GeneXpert Systems

- **GX DX Software v4.3 or higher**

Test kits (CE-IVD)

- **GXNOV-CE-10**

Additional materials required but not provided

- Single-use disposable dry swab (SDPS-120)
- Disposable transfer pipettes
- Vortex Mixer

Xpert Norovirus Kit

	Xpert Norovirus Assay
Catalogue Number	**GXNOV-CE-10**
Tests per kit	10
Reagent Sample Bottle per kit	10
Contents per test cartridge	Reagent beads
	Liquid Reagents
Kit CD	Assay Definition File (ADF)
	Instruction to import ADF
	Package Insert (PDF)
Storage	2-8 °C

Xpert Norovirus Sample Collection, Transport, and Storage

- **Sample Type:**
  - Raw or unpreserved unformed stool sample in a clean container.
- **Sample Collection:**
  - Follow your institutions guidelines for collecting samples for Norovirus testing.
- **Sample Transport and Storage:**
  - Transport at 2-8º C.
  - Store at 2-8º C for up to 2 days after the date of collection.

Good Laboratory Practice

PCR laboratory setup	• Cartridge/reagent preparation → Sample addition → Detection
Specimen and reagent storage	• Store specimens separately from reagents to prevent reagent contamination.
Equipment	• Use filtered pipette tips, when needed, for QC dilutions. • Follow the manufacturer's recommendation for calibration and maintenance of the lab equipment.

Good Laboratory Practice

Housekeeping

- Clean work surfaces with a final concentration of 1:10 dilution of household bleach and then a 70% ethanol or 70% isopropyl alcohol. Wipe work surfaces dry.
- If contamination occurs, thoroughly clean the contaminated area with 1:10 dilution of household bleach, DNA AWAY, or 3% (w/v) hydrogen peroxide and rinse thoroughly with water. Wipe work surfaces dry.

Personnel

- Wear clean lab coats and gloves.
- Change gloves between processing samples.

Lab bench area

- Clean the lab bench area routinely.
- Keep the back of the instrument dust free.

Xpert Norovirus
Kit Storage and Handling

- **Store test kits at 2-8º C. Do not use expired cartridges.**
- **Each single-use cartridge is used to process one test. Do not reuse processed cartridges.**
- **Do not open a cartridge until ready to use.**
  - **Cartridge should be run within 30 minutes after opening the lid.**
- **Avoid cross contamination during sample handling steps.**
  - **Change gloves if they come in contact with specimen or appear to be wet.**
  - **Change gloves before leaving work area and upon entry into work area.**
- **Do not use a cartridge that has been dropped or shaken after the sample has been transferred to the cartridge.**
- **Do not use a cartridge that has a damaged reaction tube.**

Norovirus Specimen Processing Swab

SCORE MARK

Cepheid Single Use Disposable Swab, Part Number SDPS-120

Xpert Norovirus – Correct Sample Amount

Too Little Sample

Right Amount

Too Much Sample

Xpert Norovirus Assay Testing Protocol

Xpert® Cartridge Preparation

- Xpert MRSA
- Xpert MRSA/SA SSTI
- Xpert C. difficile
- Xpert SA Nasal Complete
- Xpert vanA/vanB
- Xpert Norovirus

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

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

Cepheid Technical Support
US office
(888) 838-3222, Option 2
techsupport@cepheid.com

European office
+33 563 82 53 19
support@cepheideurope.com

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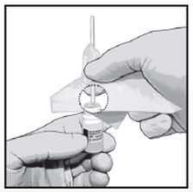
1 Obtain one Xpert cartridge and one Sample Reagent vial for each sample.

2 Insert the swab into the Sample Reagent vial.



3 Break the swab at the score mark near the opening of the vial.

4 Recap the Sample Reagent vial and vortex for 10 seconds.

5 Open the Xpert cartridge lid.

6 Aspirate all of the Sample Reagent vial contents with a disposable transfer pipette.

7 Empty the pipette into the sample chamber.

8 Close the Xpert cartridge lid.

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

Note: Do not hold the swab below the score mark. Use gauze or its equivalent to minimize the risk of contamination.

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Xpert Norovirus Protocol at a Glance

1. Place eluted sample in cartridge, then place cartridge in the instrument
2. Sample dissolves the SPC bead
3. RNA is bound to the filter
4. RNA is purified by washing steps
5. RNA is eluted from the column and mixed with RT-PCR reagent beads
6. RT step, followed by PCR amplification and detection
7. Results are ready to view and print after 90 minutes

EAT (Early Assay Termination)

- **What is it?**
  - Real-time monitoring of reaction progress
  - Termination of the reaction when the cycle threshold of a particular reaction is crossed

- **What are the benefits?**
  - Positive results are reported immediately
  - For time-critical interventions, valuable minutes are saved for patients that need it the most

Quality Control

Refer to the PI for further details

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Cepheid Assay Control Strategy

- **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.**
    - **Instrument system control: Check status**
    - **Reagent control: Probe Check**
    - **Sample processing control: SPC and/or SAC**
    - **Amplification control: SPC and/or SAC and/or IC**

Instrument System Control – Check Status

- **System control checks the optics, temperature of the module and mechanical integrity of each cartridge.**
  - **If the system controls fail, an ERROR test result will be reported.**

Reagent Control - PCC

- After sample preparation, bead reconstitution, and tube filling (prior to thermal cycling), multiple fluorescent readings are taken at different temperatures.
- The readings are compared to default settings established by Cepheid.
- The Probe Check controls for:
  - Missing Target Specific Reagent (TSR) and/or Enzyme Reagent beads, which contain all primers, probes, and internal control template
  - Incomplete reagent reconstitution
  - Incomplete reaction tube fill
  - Probe degradation
- If the Probe Check fails, an ERROR test result will be reported.

Sample Processing Controls - SPC

- The Sample Processing Control (SPC) assesses the effectiveness of the sample preparation steps, including reaction tube filling.
- SPC is armored RNA.
- The SPC controls for:
  - Missing primer/probe or enzyme beads
  - Incomplete reagent reconstitution
  - Incomplete reaction tube fill
  - Enzyme degradation
  - Sample lysis, nucleic acid extraction, and integrity of nucleic acid
  - Sample inhibition
- The SPC can be negative or positive in an analyte-positive sample.
- If the SPC fails in an analyte negative sample, an INVALID test result will be reported.

Commercially Available External Controls

ZeptoMetrix part Number	Description	Configuration
NATNOVI-ST	• Norovirus GI (positive)	**1mL**
NATNOVII-ST	• Norovirus GII (positive)	**1mL**
NATROTA-ST	• Rotavirus Wa (negative)	**1mL**
http://www.zeptoMetrix.com		

- **Other options:**
  - **Known patient positive and negative samples**
- **External controls should be used in accordance with local, state, and federal accrediting organizations, as applicable.**

Results Analysis

Refer to the PI for further details

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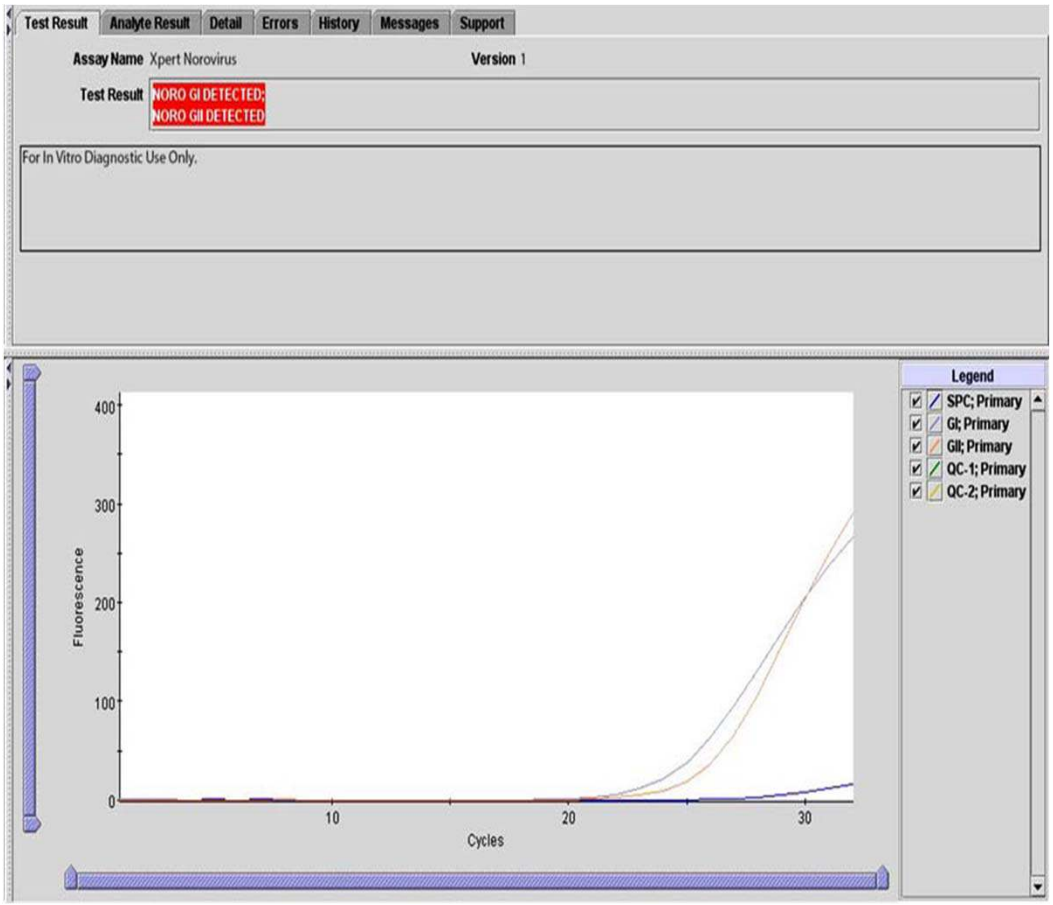
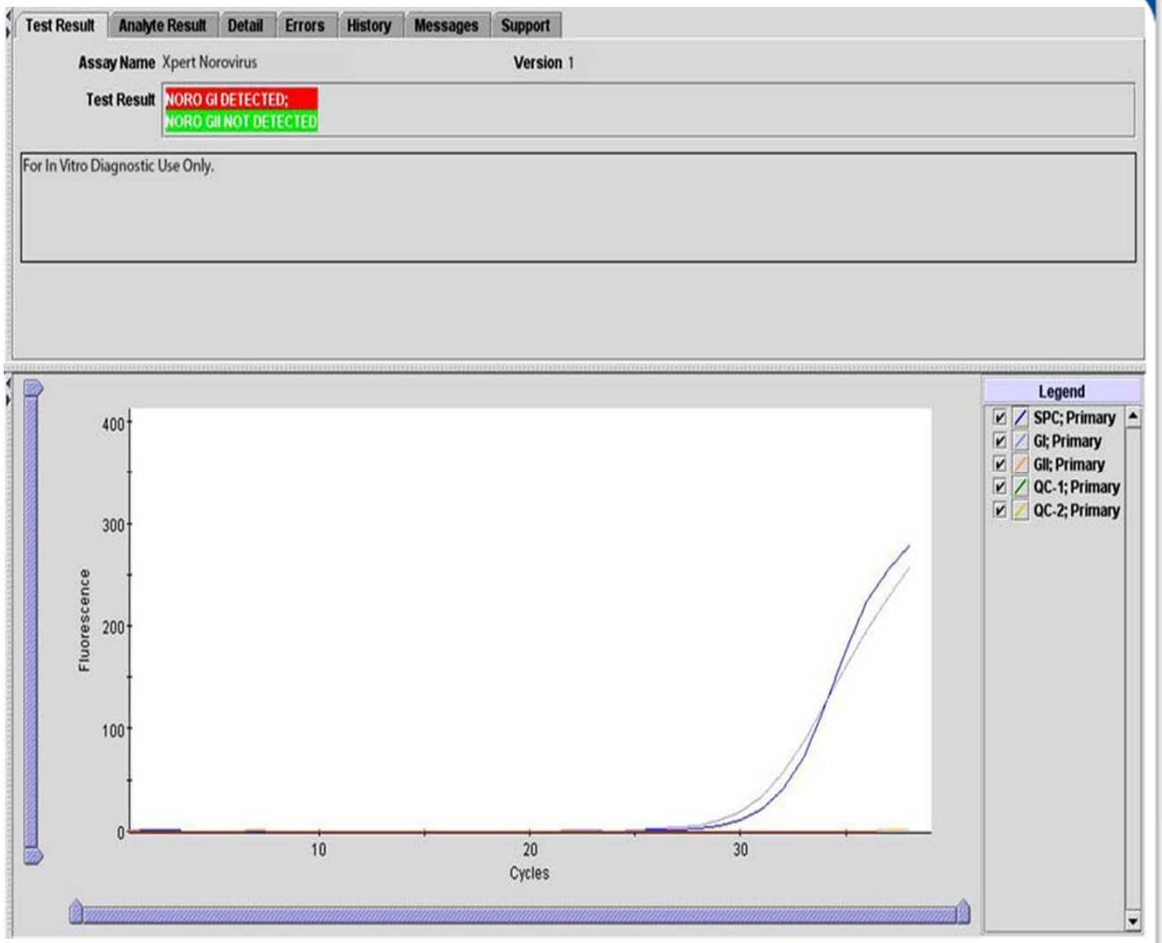
Xpert Norovirus Results: Noro GI Detected, Noro GII Detected

- **Norovirus GI and GII target RNA sequences are detected.**
- **SPC: Not applicable (NA). SPC is ignored because Norovirus target amplification can compete with this control.**
- **PCC: PASS; All probe check results pass.**



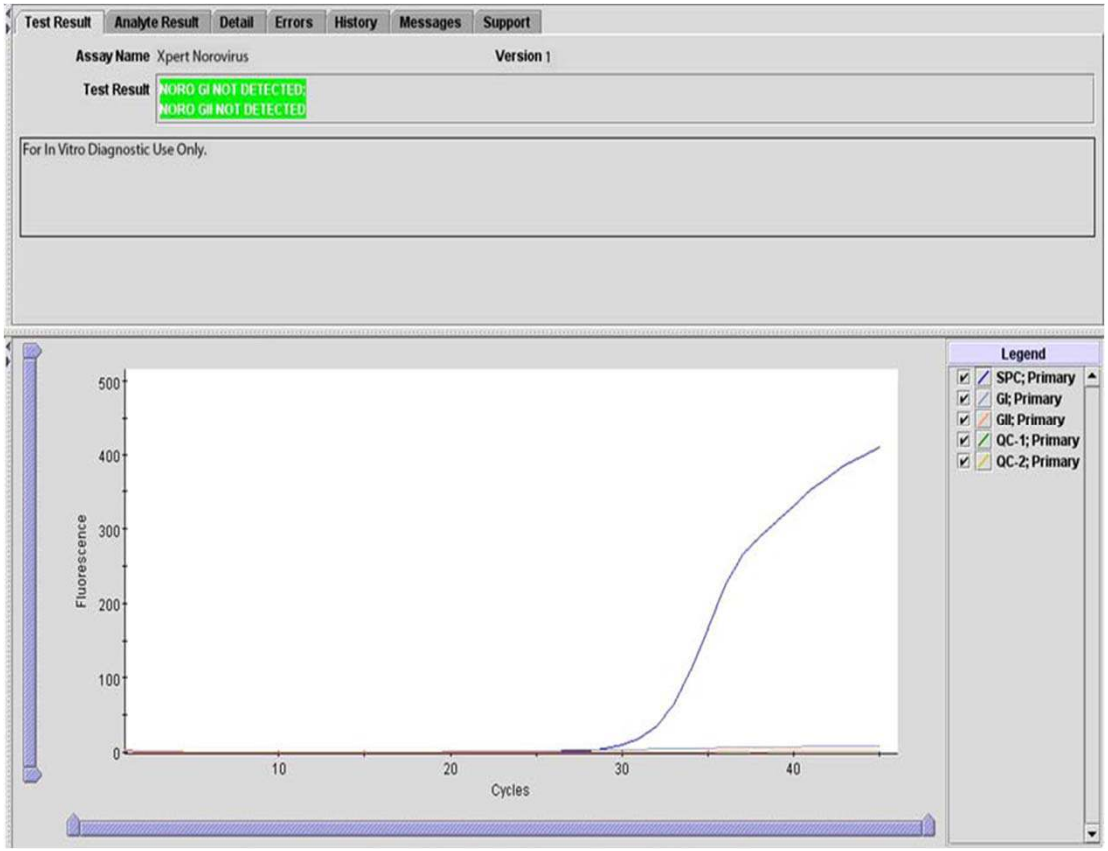
Xpert Norovirus Results: Noro GI Detected; Noro GII Not Detected

- **Norovirus GI target RNA sequences are detected.**
- **Norovirus GII RNA sequences are not detected.**
- **SPC: Pass; SPC has CT within valid range and endpoint above the endpoint minimum setting.**
- **PCC: PASS; All probe check results pass.**



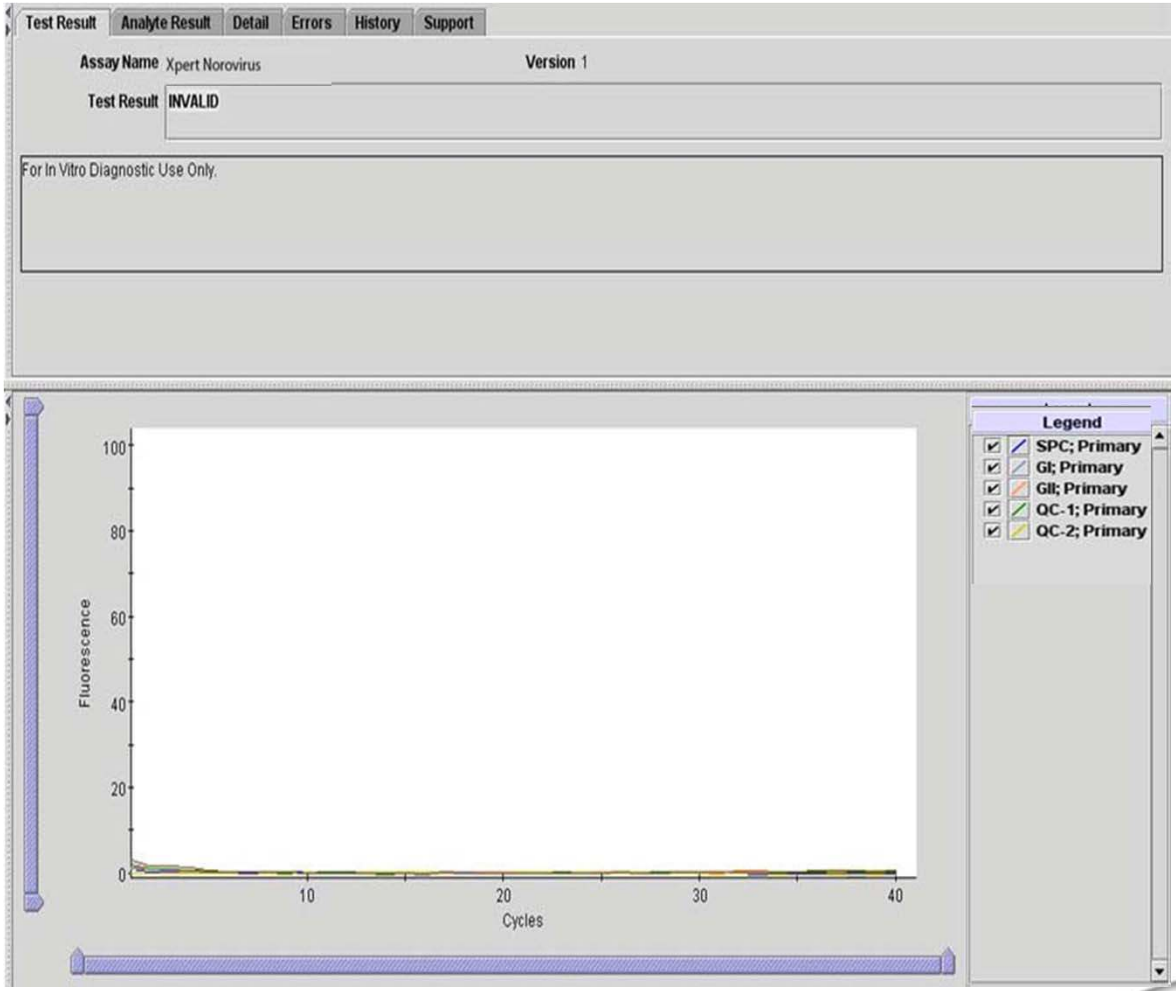
Xpert Norovirus Results: Noro GI Not Detected; Noro GII Not Detected

- **Norovirus GI and GII RNA sequences are not detected.**
- **SPC: Pass; SPC has CT within valid range and endpoint above the endpoint minimum setting.**
- **PCC: PASS; All probe check results pass.**



Xpert Norovirus Results: INVALID

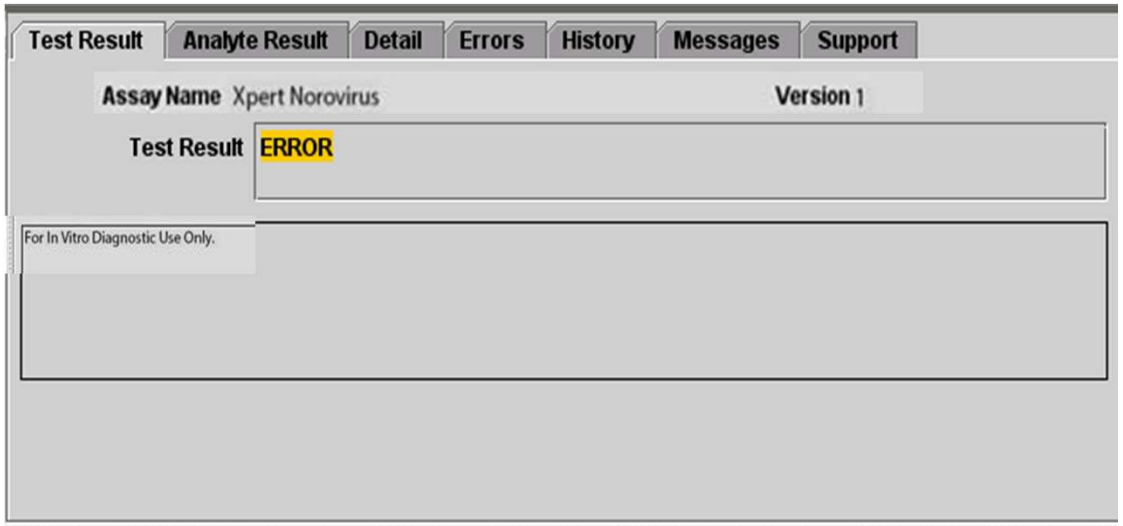
- **Presence or absence of Norovirus target RNA sequences cannot be determined. Repeat the test according to the instructions in Section 14 of the Package Insert, Retest Procedure to repeat the test.**
- **SPC target result is negative. The SPC Ct is not within valid range and fluorescence endpoint is below the minimum setting.**
- **PCC: PASS; all probe check results pass.**

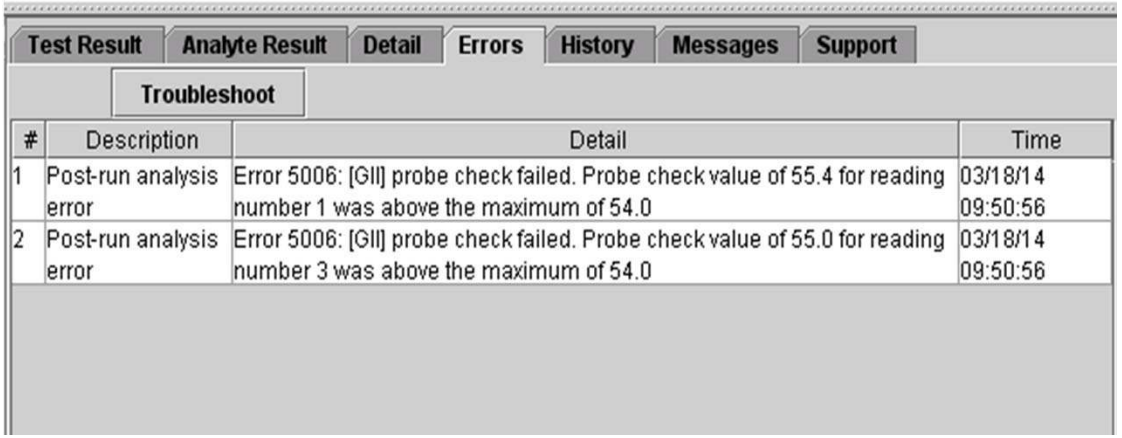


Xpert Norovirus Result: ERROR

- **Presence or absence of Norovirus target RNA sequences cannot be determined. Use the instructions in Section 14 of the Package Insert, Retest Procedure to repeat the test.**
- **SPC: NO RESULT**
- **PCC: FAIL*; all or one of the probe check results fail. The PCC probably failed because the reaction tube was filled improperly or a probe integrity problem was detected.**

*** If the probe check passed, the error is caused by a system component failure.**





No Result

Insufficient data were collected to produce a test result (for example, the operator stopped a test that was in progress).

Reasons to Repeat the Assay

- An INVALID result indicates that the sample was not properly processed, PCR was inhibited, or the sample was inadequate.
- An ERROR result indicates that the Probe Check Control failed or 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, a load error occurred, or the software was closed prematurely.

Norovirus Retest Procedure

1	**Discard used cartridge.**	
2	Obtain the leftover sample. If the leftover sample volume is insufficient, or the retest continues to return an **INVALID**, **ERROR**, or **NO RESULT**, collect a new sample.	
3	**Repeat the test with a new cartridge and sample reagent.**	
4	**Follow the Package Insert on how to run a test.**	

Factors That Negatively Affect Results

- **Improper specimen collection**
  - Performance with other collection devices and specimen types has not been assessed.
  - For assays that contain the SAC control, a specimen that does not contain human cells will result in an invalid test result.
- **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.
  - Technical error or sample mix-up can impact test results.
  - Careful compliance with the package insert is necessary to avoid erroneous results.
- **Interfering substance**
  - False negative test results or invalid results may be observed in the presence of interfering substance.
- **The number of organisms in the specimen is below the detection limit of the test**
- **Refer to Package Insert for non-determinate rate**

Interfering Substances

- **Assay interference may be observed in the presence of:**
  - **-Benzalkonium** (Antiseptic Towelettes) **greater than or equal to 0.2% w/v**
  - **-Bismuth at a concentration greater than 5% w/v**

- **Please refer to Xpert Norovirus Package Insert for data on interfering substances.**

Discussion and Q&A

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Technical Support

- **Cepheid provides technical support in the field, on the phone, by fax, and by email.**

- **North America:**
  - **Phone: +1 (888) 838-3222**
  - **Fax: +1 (408) 716-2550**
  - **Email: techsupport@cepheid.com**

- **Europe, Africa, Middle-East, Indian Peninsula:**
  - **Phone:**
    France: + 33 5 63 82 53 19
    Germany : + 49 69 710 480 480
    UK : + 44 3303 332533
    South Africa : +27 11 234 9636
    Australia, New Zealand : +61 1800 107 884
  - **Fax: +33 563 82 53 01**
  - **Email: support@cepheideurope.com**

Discussion and Q&A