

Assay Technical Training

Xpert[®] HPV

For CE-IVD Use Only

Cepheid Training Center



Training Agenda

- **Xpert HPV**
 - Clinical utility
 - Reagents
 - Sample collection
 - Kit storage and handling
 - Preparing the cartridge
 - Quality Controls
 - Results Analysis
- **Discussion**



Training Objectives

- **At the end of the training, user will be able to:**
 - Properly store and handle the Xpert[®] HPV cartridge kit and sample collection kits
 - Follow proper laboratory safety precautions
 - Collect and transport the appropriate specimen
 - Prepare a cartridge and run the assay
 - Report the various software generated results
 - Understand the Xpert HPV control strategy

The Cepheid Solution



- Simultaneous detection
 - High Risk HPV types
- On-board controls for each individual sample
 - Probe Check Control (PCC)
 - Sample Adequacy Control (SAC)
- Results in approximately
 - 60 minutes
- Closed cartridge system minimizes risk of contamination
- On-demand results 24/7
- Random access

Intended Use

The Xpert HPV Assay is a **qualitative *in vitro*** test for the detection of the **E6/E7 region** of the viral DNA genome from **14 high risk HPV types in a single analysis**. Xpert HPV specifically identifies types **HPV16** and **HPV 18/45** in two distinct detection channels, and reports 11 **other High-risk types** (31, 33, 35, 39, 51, 52, 56, 58, 59, 66 and 68) in a pooled result.

- **Specimen**

- Cervical cells collected in PreservCyt® Solution

Specimens pre-treated with Glacial Acetic Acid (GAA) have also been validated for use with the Xpert HPV Assay.

- Detection of HPV16 and HPV 18/45 and 11 other High-risk types

	HPV Types Detected
HPV 16	HPV 16
HPV 18_45	HPV {18 and 45}
P3	HPV {31,35,33,52,58}
P4	HPV {51,59}
P5	HPV {39,68,56,66}

Specimen, Targets and Probes

- **Specimen**

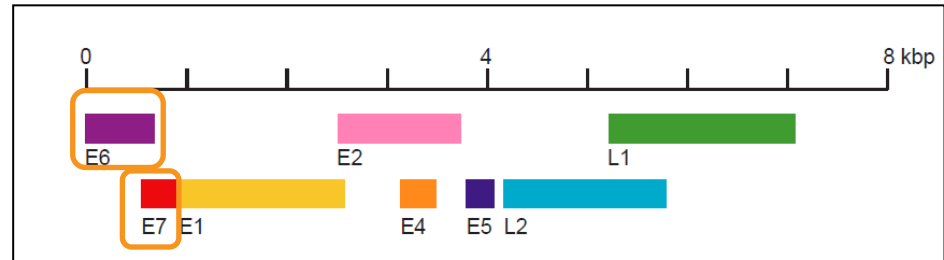
- Cervical cells collected in PreservCyt® Solution
- Specimens pre-treated with Glacial Acetic Acid (GAA) have also been validated for use with the Xpert HPV Assay.

- **Target**

- **High Risk HPV types**

- **Probes**

- 1 probe binds to the SAC
- Remaining probes bind depending on the presence of HR HPV types detected in the patient sample



Adapted from Expert Reviews in Molecular Medicine © Cambridge University Press ISSN 1462-3994

Xpert HPV Requirements

GeneXpert Systems

- GeneXpert Software **v4.3a** or higher
- Infinity Software **v6.1** or higher

Test Kits (CE-IVD)

- GXHPV-CE-10

Sample Collection

- Sample Collection Kit: PreservCyt Solution (Hologic Corp.) #PRESERVCYT50
- Endocervical Brush #CERVEXBRUSH25

Other materials

- Personal Protective Equipment (PPE)
- 1:10 Bleach
- 70% ethanol or denatured ethanol
- Vortex

Optional

- Uninterruptible Power Supply /Surge Protector
- Printer

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)

Kit handling



Xpert HPV Kit Contents

Catalogue Number	GXHPV-CE-10
Cartridges Per Kit	10
Kit CD	Assay Definition File (ADF)
	Assay Import Instructions
	Package Insert (PDF)
Transfer Pipettes	10
Storage	2-28 ° C



Cartridges contain chemically hazardous substances-please see Package Insert and Safety Data Sheet for more detailed information.

Xpert HPV Kit Storage and Handling

- Store the Xpert Assay cartridges and reagents at 2–28°C
- Follow your institution's safety procedures for working with chemicals and handling biological samples
- Do not use Collection Reagent tubes that have not been validated by Cepheid
- Open the Assay cartridge lid only when adding the Sample, close the lid and proceed with processing



Warnings and Precautions

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



Warnings and Precautions

- 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.

Xpert HPV Assay Limitations

- The Xpert HPV Assay has only been validated with cervical specimens collected in **PreservCyt Solution** using either a **broom-like device** or an **endocervical brush/spatula** combination.
- Assay interference may be observed in the presence of: whole blood, peripheral blood mononuclear cells, *Candida albicans* or thick vaginal creams.
- The effects of other potential variables such as vaginal discharge, use of tampons, douching, and specimen collection variables have not been determined.
- The performance of the Xpert HPV Assay has not been evaluated for HPV-vaccinated individuals.

For detailed information, refer to the current Package Insert

Specimen Collection, Storage and Transport

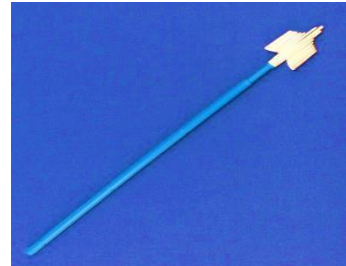


Xpert HPV Sample Collection

- **Sample Type:**
 - Cervical specimens in PreservCyt® Solution



- **Sample Collection:**
 - Follow the manufacturer's instructions for collecting cervical specimens using a broom-like device OR brush/spatula with detachable heads





or



<https://healthdxs.com/en/> for more information

Specimen Transport and Storage

	Prior to testing	Temperature (°C)	Storage Time
	Cervical specimens collected in PreservCyt Solutions		Up to 6 months

- Cervical specimens collected in PreservCyt Solution can be transported at 2–30 °C.
Transportation of HPV specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.
- Cervical specimens collected in PreservCyt Solution may be stored at 2–30 °C for up to six months after the date of collection.

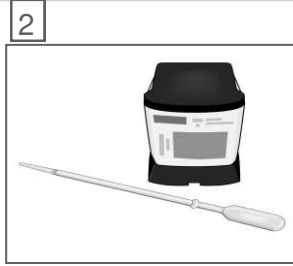
Cartridge Preparation



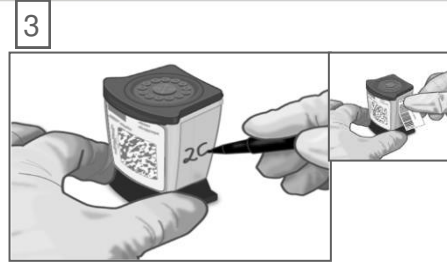
Xpert HPV- Cartridge Preparation



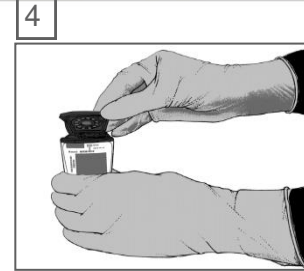
Obtain an HPV cervical sample collected in PreservCyt Solution



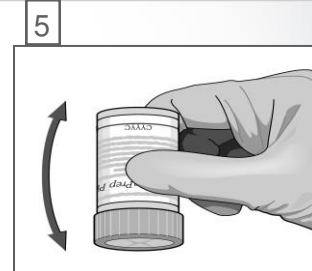
Take one Xpert HPV cartridge and the provided transfer pipette



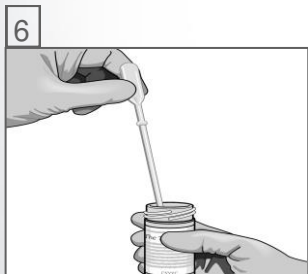
Label the side of the cartridge with the same ID as the sample ID



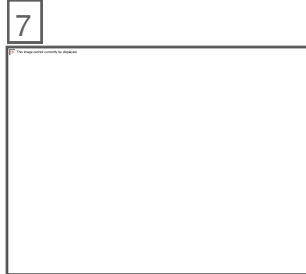
Open the cartridge lid



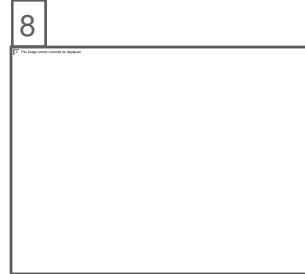
Gently invert the transport tube 8-10 times to mix
Or vortex for 5 seconds at half-speed



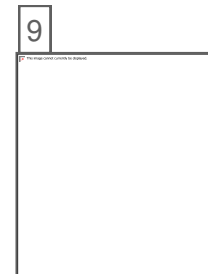
Pipette at least 1 mL of the sample using the provided pipette*



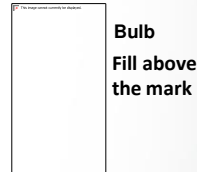
Slowly empty the pipette into the sample chamber of the cartridge



Close the lid firmly



Start the test on your GeneXpert Instrument within the recommended time



*HPV Assay Transfer pipette

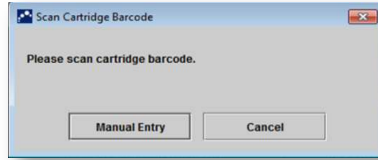
Run a Test

1 Create Test

GeneXpert



2 Scan barcode messages: Cartridge/ Patient and/or Sample ID

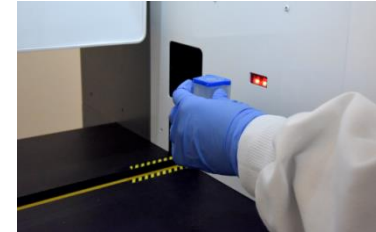
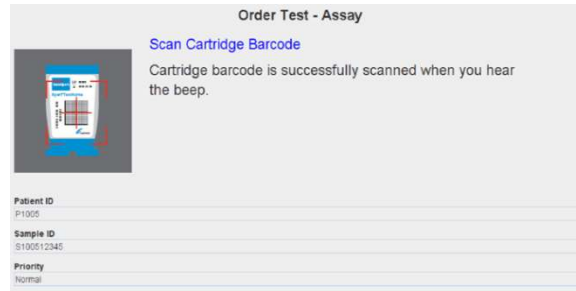


*By default, do not click on
Manual Entry or Cancel*

3 Scan the cartridge



GeneXpert
Infinity



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

Create a Test

4 Complete the fields as required

5 Select the appropriate Assay Protocol (ADF) from the drop-down list

6 The module is selected automatically
DO NOT CHANGE IT!!!

7 Click on Start Test

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

Name	Version
Xpert HPV HR_16_18-45	1
Xpert HPV 16_18-45	1
Xpert HPV HR	1
Xpert HPV HR_16_18-45	1



Create a Test on Xpert Dx Software

4 Complete the fields as required

Order Test - Test Information

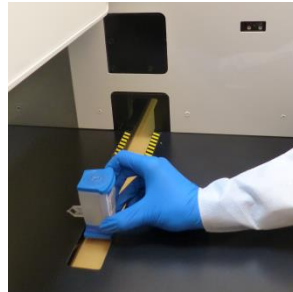
Patient ID patientid	
Sample ID sampleid	
Last Name patient	First Name id
Assay* Xpert HPV	
Reagent Lot ID* 12102	Cartridge S/N* 282769448
Expiration Date* 2018/11/04	Priority Normal
Test Type Specimen	
Sample Type Other	Other Sample Type
Notes	



5 Select the appropriate Assay Protocol (ADF) from the drop-down list

6 Click on SUBMIT

7 Place the cartridge into the conveyor belt



Automated Xpert Protocol



Quality Controls



Assay Control Strategy

CONTROL

- **Xpert HPV Quality Controls**

- 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
 - Sample Adequacy Control (SAC)
 - Probe Check Controls (PCC)

- **Probe Check Controls (PCC)**

- Before the PCR step fluorescent signal is measured on all probes and compared with pre-established factory settings to monitor
 - bead rehydration
 - reaction tube filling
 - probe integrity
 - dye stability

- **Sample Adequacy Control SAC**

- HMBS (Hydroxymethylbilane synthase)
 - Ensures that human cells have been added in the sample chamber of the cartridge
 - If negative can indicate poor sampling or sample mixing
 - Must be positive in a negative sample
 - Can be positive or negative in a positive sample

Commercially Available External Controls

AcroMetrix® <http://www.lifetechnologies.com/acrometrix>

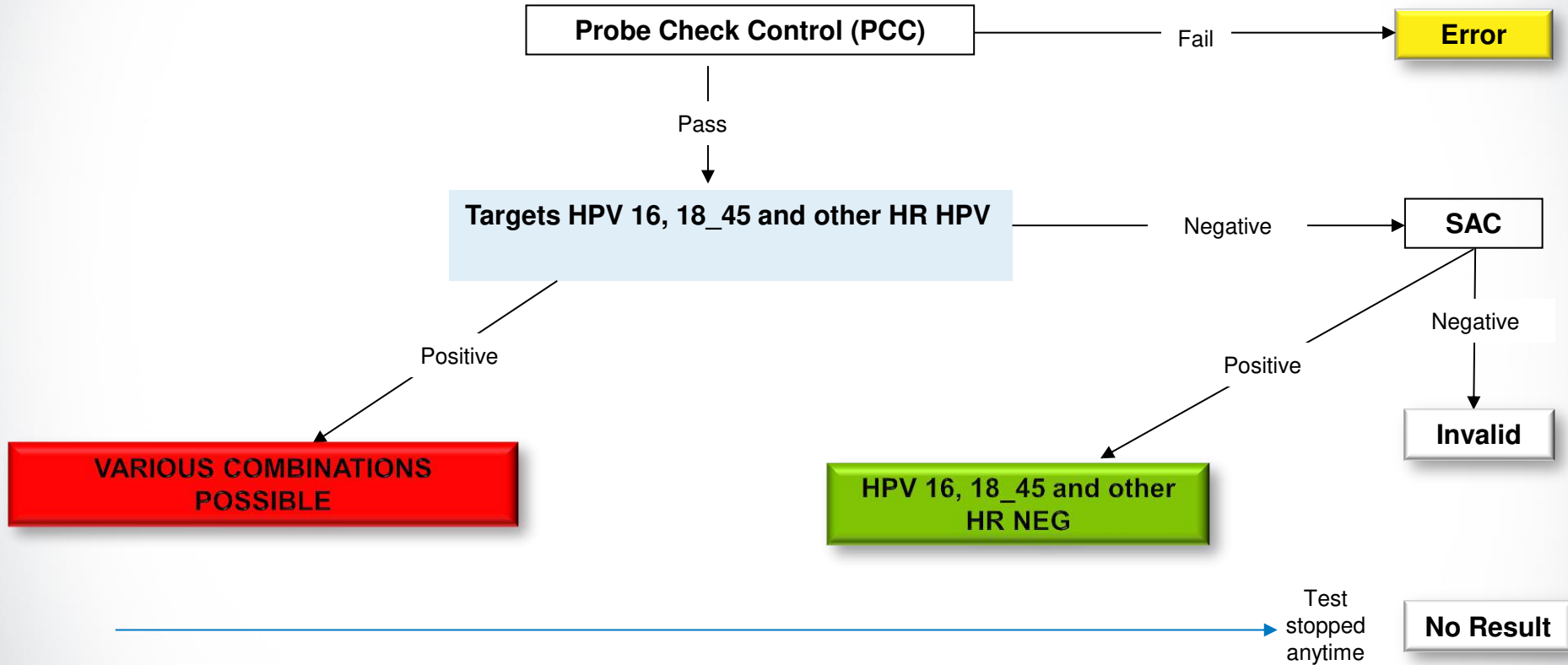
Part Number	Description	Configuration	Storage
950075	HPV 16 positive	5 x 4mL/box	2-8°C
950076	HPV 18 positive	5 x 4mL/box	2-8°C
950078	Negative for HPV	5 x 4mL/box	2-8°C

- Many other vendors for quality control material are also available than the one outlined above.
- External controls should be used in accordance with local, state accrediting organizations, as applicable

Result Interpretation



Result Interpretation Algorithm



Xpert HPV Result Interpretation

Displayed Results	HPV 16	HPV 18_45	Other HR HPV
HPV16 POS: HPV 18_45 NEG: OTHER HR HPV NEG	+	-	-
HPV16 POS: HPV 18_45 POS: OTHER HR HPV NEG	+	+	-
HPV16 NEG: HPV 18_45 NEG: OTHER HR HPV NEG	-	-	-
HPV16 NEG: HPV 18_45 POS: OTHER HR HPV NEG	-	+	-
HPV16 NEG: HPV 18_45 NEG: OTHER HR HPV POS	-	-	+
HPV16 POS: HPV 18_45 POS: OTHER HR HPV POS	+	+	+

HPV 16 POS

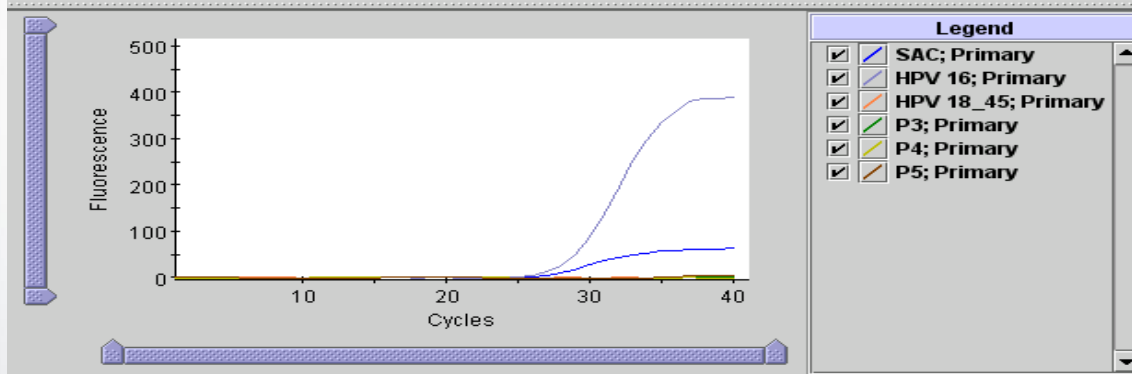
Test Result	Analyte Result	Detail	Errors	History	Support
Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result	
SAC	28.7	63	NA	PASS	
HPV 16	28.0	388	POS	PASS	
HPV 18_45	0.0	4	NEG	PASS	
P3	0.0	1	NEG	PASS	
P4	0.0	-1	NEG	PASS	
P5	0.0	5	NEG	PASS	

HPV 16 POS;
HPV 18_45 NEG;
OTHER HR HPV NEG

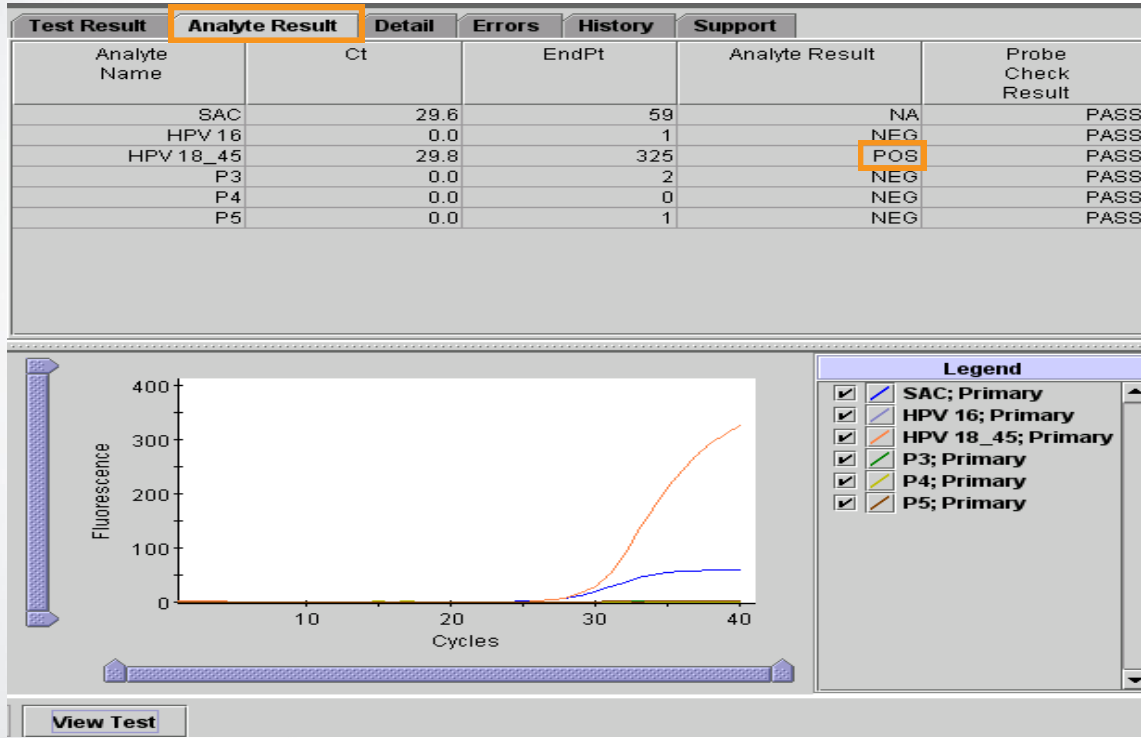
-HPV16 target DNA sequence has a Ct within the valid range and a fluorescence endpoint above the threshold setting.

-SAC: SAC is not applicable because the HPV target amplification can compete with this control.

-Probe Check: PASS



HPV 18_45 POS



HPV 16 NEG;
HPV 18_45 POS;
OTHER HR HPV NEG

-HPV18_45 target DNA has a Ct within the valid range and a fluorescence endpoint above the threshold setting.

-SAC: SAC is not applicable because the HPV target amplification can compete with this control.

-Probe Check: PASS

Other HR HPV POS

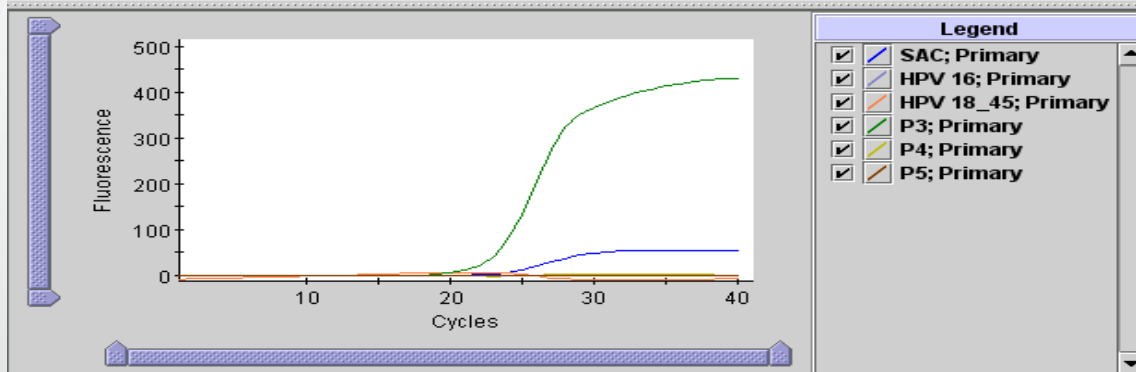
Test Result	Analyte Result	Detail	Errors	History	Support
Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result	
SAC	25.4	54	NA	PASS	
HPV 16	0.0	0	NEG	PASS	
HPV 18_45	0.0	-7	NEG	PASS	
P3	21.4	431	POS	PASS	
P4	0.0	1	NEG	PASS	
P5	0.0	0	NEG	PASS	

HPV 16 NEG;
HPV 18_45 NEG;
OTHER HR HPV POS

-Other HR HPV target DNA sequences has a Ct within the valid range and a fluorescence endpoint above the threshold setting.

-SAC: SAC is not applicable because the HPV target amplification can compete with this control.

-Probe Check: PASS



HPV NEG

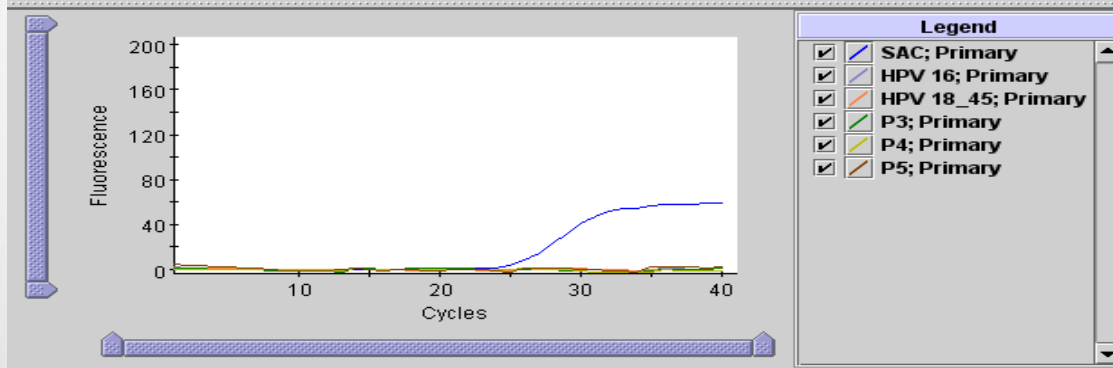
Test Result	Analyte Result	Detail	Errors	History	Support
Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result	
SAC	27.1	59	PASS	PASS	
HPV 16	0.0	2	NEG	PASS	
HPV 18_45	0.0	1	NEG	PASS	
P3	0.0	1	NEG	PASS	
P4	0.0	-1	NEG	PASS	
P5	0.0	2	NEG	PASS	

HPV 16 NEG;
HPV 18_45 NEG;
OTHER HR HPV NEG

-The targeted HPV DNA sequences have a Ct value that is not within the valid range and/or a fluorescence endpoint below the threshold setting.

-SAC: SAC has a Ct value within the valid range

- Probe Check: PASS



Troubleshooting



Factors That Negatively Affect Results

- Improper specimen collection
 - Performance with other collection devices and specimen types has not been assessed.
- Improper transport or storage of collected specimen
 - 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.
- Interfering substances
 - False negative results or invalid results may be observed in the presence of interfering substances.

INVALID Result

INVALID

Test Result	Analyte Result	Detail	Errors	History	Support
Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result	
SAC	0.0	-1	FAIL	PASS	
HPV 16	0.0	0	INVALID	PASS	
HPV 18_45	0.0	0	INVALID	PASS	
P3	0.0	0	INVALID	PASS	
P4	0.0	-3	INVALID	PASS	
P5	0.0	-1	INVALID	PASS	

Presence or absence of HPV target cannot be determined.

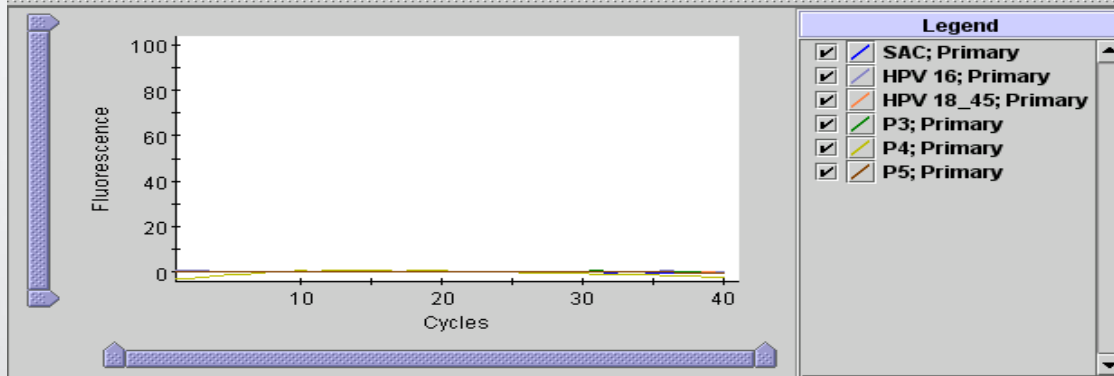
- SAC:FAIL The SAC does not meet the acceptance criteria
- Probe Check: PASS

Possible Causes

- Improper sample collection
- Incorrect sample preparation
- Improper storage of the cartridges
- Inefficient sample processing in cartridge
- Presence of interfering substances in the sample

Solution

- Repeat the test with a new cartridge and new sample



Xpert HPV Interference

- Assay Interference

A total of 16 endogenous and exogenous substances were evaluated at specific concentrations.

Assay interference may be observed in the presence of:

Substance	Concentration
Vagisil anti-itch cream	0.25% w/v
Thick creams	>0.25% w/v
Whole blood	0.25% v/v
Vagi-Gard Moisturizing Gel	0.5% w/v
C.albicans	$\geq 1 \times 10^8$ cells/mL

w=weight; v=volume

For detailed information, refer to the current Package Insert

ERROR Result

ERROR

The screenshot shows a software interface with several tabs: **Test Result**, **Analyte Result**, **Detail**, **Errors**, **History**, and **Support**. The **Test Result** tab is active, displaying the following information:

- Assay Name:** Xpert HPV HR
- Version:** 1
- Test Result:** ERROR (highlighted with an orange circle)
- For In Vitro Diagnostic Use Only.**
- <No Data Available>**

- The presence or absence of HPV cannot be determined

Possible Causes

If Probe Check: FAIL

- Improper Sample collection
- Incorrect Sample volume added to the cartridge

If Probe Check: PASS

- Check the GeneXpert System module

Solution

- Repeat the test with a new cartridge

NO RESULT

NO RESULT

The screenshot shows a software interface with several tabs: 'Test Result', 'Analyte Result', 'Detail', 'Errors', 'History', and 'Support'. The 'Test Result' tab is active. Below the tabs, the 'Assay Name' is 'Xpert HPV HR' and the 'Version' is '1'. The 'Test Result' field displays 'NO RESULT', which is circled in orange. Below this field, there is a large empty box with the text 'For In Vitro Diagnostic Use Only.' at the top and '<No Data Available>' at the bottom.

- The presence or absence of HPV cannot be determined.
- NO RESULT indicates that insufficient data were collected.

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

Re-test Procedure

1



Discard used cartridge

Follow your institution's safety guidelines for disposal of cartridges

2



Obtain the residual sample, mix according to Package Insert

If the leftover sample volume is insufficient, or the retest continues to return an INVALID, ERROR, or NO RESULT, collect a new sample

3



Obtain a new cartridge

Label appropriately as retest on the new cartridge

Process the sample per the package insert

4



Run the test on the GeneXpert System



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 using the following link <http://www.cepheid.com/us/support> - *Create a Support Case*

Region	Telephone	Technical Support Email
US	+ 1 888 838 3222	techsupport@cepheid.com
Australia and New Zealand	+ 1800 107 884 (AU) + 0800 001 028 (NZ)	techsupportANZ@cepheid.com
Brazil and Latin America	+ 55 11 3524 8373	latamsupport@cepheid.com
China	+ 86 021 5406 5387	techsupportchina@cepheid.com
France	+ 33 563 825 319	support@cepheideurope.com
Germany	+ 49 69 710 480 480	support@cepheideurope.com
India, Bangladesh, Bhutan, Nepal, and Sri Lanka	+ 91 11 48353010	techsupportindia@cepheid.com
Italy	+ 39 800 902 567	support@cepheideurope.com
South Africa	+ 27 861 22 76 35	support@cepheideurope.com
United Kingdom	+ 44 3303 332 533	support@cepheideurope.com
Other European, Middle East, and African countries	+ 33 563 825 319 + 971 4 253 3218	support@cepheideurope.com
Belgium, Netherlands and Luxembourg	+33 563 825 319	support@cepheideurope.com
Other European, Middle East, and African Countries	+ 33 563 825 319 + 971 4 253 3218	support@cepheideurope.com
Other countries not listed	+ 1 408 400 8495	techsupport@cepheid.com



Thank You.



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