WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: Xpert® HPV
WHO reference number: PQDx 0268-070-00

Xpert® HPV with product code **GXHPV-CE-10** manufactured by **Cepheid AB**, **CE marked regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 21 December 2017.

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 high risk Human Papillomavirus (HPV) in patient specimens. The test carries out multiplexed amplification of target DNA by real-time Polymerase Chain Reaction (PCR) of 14 high risk HPV types in a single analysis. Xpert® HPV specifically identifies types HPV 16 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. Specimens are limited to cervical cells collected in PreservCyt® Solution (Hologic Corp.). Cervical specimens collected in PreservCyt Solution that have been pretreated with Glacial Acetic Acid (GAA) to lyse excess red blood cells for cytology review have also been validated for use with the Xpert® HPV assay.

Indications for the Xpert® HPV assay:

• The Xpert® HPV assay can be used with a Pap specimen to assess the presence or absence of high risk HPV types. This information, together with the physician's assessment of the patient's medical history, other risk factors, and professional guidelines, may be used to guide patient management.

• The Xpert® HPV assay can be used with a Pap specimen to assess the presence or absence of HPV genotypes 16 and 18/45.

This information, together with the physician's assessment of the patient's medical history, other risk factors, and professional guidelines, may be used to guide patient management.

Assay description:
The Xpert® HPV assay is an automated test for qualitative detection and differentiation of HPV DNA. The assay is performed on Cepheid GeneXpert Instrument Systems.

GeneXpert Instrument Systems automate and integrate sample processing, cell lysis, purification, nucleic acid amplification, and detection of the target sequences in clinical samples by using real-time PCR. The systems consist of an instrument, personal computer,

and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable GeneXpert cartridges that hold the PCR reagents, house the sample, and carry out the PCR processing. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, refer to the appropriate GeneXpert Dx System Operator Manual or the GeneXpert Infinity System Operator Manual.

The Xpert® HPV Assay includes reagents for the detection of high risk HPV. The Xpert® HPV Assay is designed for use with cervical specimens collected in PreservCyt with either a broom-like device or an endocervical brush/spatula combination. Cervical specimens pretreated with certain Glacial Acetic Acid (GAA) methods may also be used. Cervical specimens collected in PreservCyt Solution have been validated for use with the Xpert HPV Test. Follow the manufacturer's instructions for collecting cervical specimens.

A Sample Adequacy Control (SAC) and a Probe Check Control (PCC) are also included in the cartridge. SAC reagents detect the presence of a single copy human gene and monitor whether the specimen contains adequate numbers of human cells to carry out a qualitative assessment of HPV status. The PCC verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The six color channels contain primers and probes for the detection of specific genotypes or pooled results as follows: “SAC; Primary” for the Sample Adequacy Control, “HPV 16; Primary” for HPV 16, “HPV 18_45; Primary” for the HPV 18/45 pooled result, “P3; Primary” for the pooled result of any of HPV types 31, 33, 35 52, or 58, “P4: Primary” for the pooled result of either of HPV types 51 or 59, and “P5; Primary” for the pooled result of any of HPV types 39, 56, 66 or 68.

Test kit contents:

Component	**10 tests (product code GXHPV-CE-10)**
Xpert® HPV assay cartridges with integrated reaction tubes	10
Disposable 1 mL transfer pipettes	1 bag of 10 per kit
CD with Assay Definition Files (ADF) and instructions for use	1

Instrumentation:

Product name	**Product code**
GeneXpert® Dx (including barcode scanner and operator manual)	GXI-1-L, GXI-1-D, GXII-1-L, GXII-1-D, GXII-2-L, GXII-2-D, GXIV-1-L, GXIV-1-D, GXIV-2-L, GXIV-2-D, GXIV-3-L, GXIV-3-D, GXIV-4-L, GXIV-4-D, GXXVI-4-L, GXXVI-4-D, GXXVI-8-L, GXXVI-8-D,

	GXXVI-12-L, GXXVI-12-D, GXXVI-16-L, GXXVI-16-D
GeneXpert® Infinity-48s (including barcode scanner and operator manual)	INFINITY48-16, INFINITY48-16-EUROPE, INFINITY48-24, INFINITY48-24-EUROPE, INFINITY48-32, INFINITY48-32-EUROPE, INFINITY48-40, INFINITY48-40-EUROPE, INFINITY48-48, INFINITY48-48-EUROPE
GeneXpert® Infinity-80 (including barcode scanner and operator manual)	INFINITY80-16, INFINITY80-16-230V, INFINITY80-24, INFINITY80-24-230V, INFINITY80-32, INFINITY80-32-230V, INFINITY80-40, INFINITY80-40-230V, INFINITY80-48, INFINITY80-48-230V, INFINITY80-56, INFINITY80-56-230V, INFINITY80-64, INFINITY80-64-230V, INFINITY80-72, INFINITY80-72-230V, INFINITY80-80, INFINITY80-80-230V
GeneXpert Software Version 4.3 or higher, and barcode scanner or GeneXpert Infinity system with Xpertise software version 6.1 or higher	GX4.0SWKIT and XPERTISE-G2-SWKIT

Items required but not provided:

Item
Consumables: Bleach 70% Ethanol Disposable gloves Specimen collection material: • PreservCyt® Solution (Hologic Corp.) • Broom-like device or an endocervical brush/spatula
Equipment: Printer

Storage:
The test kit should be stored at 2-28 °C.

Shelf-life upon manufacture:
18 months.

Warnings/limitations:
N/A

Summary of WHO prequalification assessment for : Xpert® HPV

	Date	Outcome
PQ listing	21 December 2017	
Dossier assessment	1 August 2017	MR
Site inspection(s) of quality management system	23 and 25 June 2015 29 and 30 July 2015	MR
Product performance evaluation	12 December 2017	MR

N/A: Not applicable

Prioritization for prequalification
Based on the established criteria, Xpert® HPV was given priority for WHO prequalification.

Dossier assessment
Cepheid AB submitted a product dossier for Xpert® HPV as per the "Instructions for compilation of a product dossier" (PQDx_018 v1). The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO.

Commitments for prequalification:
1. Shipping stability: Cepheid AB will provide data to WHO demonstrating the ability of the product to remain stable in conditions of extreme humidity relevant to where this product may be used. An interim report should be submitted by 1 December 2018. The final report should be submitted by no later than 31 May 2019.

2. Robustness studies: Cepheid AB will provide data to WHO demonstrating robustness under conditions of extreme humidity. An interim report should be submitted by 1 December 2018. The final report should be submitted by no later than 31 May 2019.

3. Clinical evidence - clinical and diagnostic sensitivity and specificity: Results of additional studies performed in countries with populations and public health policies for the clinical management of cervical cancer screening equivalent to those found in the countries where the product will be actually supplied should be submitted to WHO. An interim report should be submitted by 1 December 2018. The final report should be submitted by no later than 1 December 2019.

WHO will follow-up on implementation of these commitments at the next re-inspection.

4. Revision to the instructions for use as per WHO requests to be submitted as a change notification by 30 March 2018.

Based on the product dossier screening and assessment findings, the product dossier for Xpert® HPV meets WHO prequalification requirements.

Manufacturing site inspection
In accordance with the WHO procedure, an inspection was conducted at the following sites of manufacture:

- Röntgenvägen 5, SE-171 54 Solna (Stockholm), Sweden, between 23 and 25 June 2015 where GeneXpert Dx, GeneXpert Infinity-48, GeneXpert Infinity-48s, GeneXpert Infinity-80, Xpert® HIV-1 Qual Assay with GeneXpert Dx, GeneXpert Infinity-48s, and GeneXpert Infinity-80 Infinity-80 (PQDx 0259-070-00) and Xpert® HCV Viral Load with GeneXpert Dx, GeneXpert Infinity-48s, and GeneXpert Infinity-80 (PQDx 0260-070-00) were reviewed; and
- 904 Caribbean Drive, Sunnyvale 94089-1189, California, USA and 1339 Moffet Park Drive, Sunnyvale 94089, California, USA, between 29 and 30 July 2015 where GeneXpert Dx, GeneXpert Infinity-48s and GeneXpert Infinity-80 were reviewed;

as per the "Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics" (PQDx_014 v1).

The inspection found that the manufacturer had an acceptable quality management system and good manufacturing practices in place that ensured the consistent manufacture of a product of good quality.

The manufacturer's responses to the nonconformities found at the time of the inspection were accepted 8 August 2015 and 23 May 2016.

The scope of this inspection did not include the Xpert® HPV. However, there is sufficient evidence of the adequacy of the manufacturer's QMS to meet the ISO 13485 and WHO PQ requirements for the design and manufacture of IVDs for HIV and HCV based on the inspection conducted in 2015. On the basis of the similarities of the Xpert® HPV with the Cepheid products prequalified under this inspection, and assuming that no significant changes have been introduced since 2015, it is recommended that the existing QMS evidence be used to support the inspection component of the assessment process for the Xpert® HPV assay.

Commitments for prequalification:
The manufacturer agrees to a re-inspection in the first half of 2018 so that the fulfillment of the WHO requirements can be confirmed. In addition, specific dossier related findings with respect to in-use stability will be followed up at the time.

Based on the above, the quality management system for Xpert® HPV meets WHO prequalification requirements.

Product performance evaluation
Xpert® HPV was evaluated by the Scottish Human Papilloma Virus Reference Laboratory Edinburgh, NHS Lothian, Scotland, from 24 April to 10 May 2017.

The Xpert® HPV is a qualitative *in vitro* test for the detection of the E6/E7 region of the viral DNA genome from high risk Human Papillomavirus (HPV) in patient specimens. A volume of 1000µl of specimens needed to perform the assay. This type of assay does not require additional laboratory equipment and can be performed in laboratories with limited facilities.

Analytical specimens:
In this evaluation the LOD for HPV genotype 16 was estimated to be 2903 IU/ml (95% fiducial limits: 1081-20463). The limit of detection for HPV genotype 18 was estimated to be 50493 IU/ml (95% fiducial limits: 10711-5267264).

Clinical specimens:
In this limited performance evaluation, we found, an initial positive percent agreement (95% CI) of 82.76% (73.16-90.02) and an initial negative percent agreement (95% CI) of 97.63% (95.54-98.91) compared to the benchmark results.

The final positive percent agreement and negative percent agreement (95% CI) were 88.51% (79.88-94.35) and 98.68% (96.95-99.57).

In this study, the error rate was 0.52% during the analytical testing (limit of detection) and 7.17 % during the testing of clinical specimens.

Protocol limitations:

- While WHO standards can provide insight into analytical sensitivity of the assay, it is noted that these are not bio-specimens validated for formal calibration purposes, nor do they represent or resemble the biological matrix of clinical specimens for which the assay under evaluation was validated. These aspects have implications for the comparison between the LOD reported in the instructions for use and that observed in this study and should be taken into account if there is disagreement.

- This laboratory evaluation protocol was designed to verify the ability for the assays under evaluation to detect HPV DNA (in line with the manufacturer's stated intended use). Pathology data were not available given the setting/infrastructure where patient sampling occurred. Consequently, clinical performance relative to CIN2+ could not be assessed nor could the level of *clinically relevant* discordance between the test under evaluation and the benchmark test.

Performance characteristics	
Analytical performance	
Limit of Detection	HPV genotype 16: 2903 IU/ml (95% fiducial limits: 1081-20 463). HPV genotype 18: 50 493 IU/ml (95% fiducial limits: 10 711-5 267 264)

Clinical performance		
	Initial (95% CI)	**Final (95% CI)**
Positive percent agreement %	82.76% (73.16-90.02)	88.51% (79.88-94.35)
Negative percent agreement %	97.63% (95.54-98.91)	98.68% (96.95-99.57)
Invalid rate	0.52% during the analytical testing (limit of detection) and 7.17 % during the testing of clinical specimens.	

Key operational characteristics	
Validated specimen types	Cervical cells collected in PreservCyt® Solution (Hologic Corp.)
Number of steps	4 from addition of the specimen to result.
Time to result	Approximately 60 minutes
In-use stability of reagents	Reagents are all contained within the cartridge.

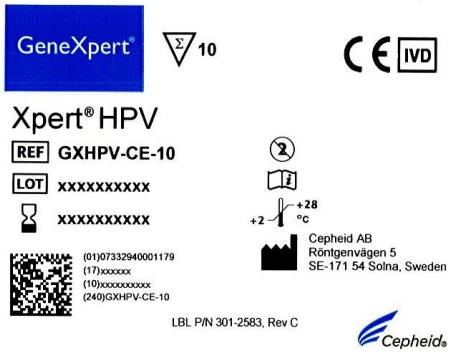
Labelling

1. **Labels**
2. **Instructions for use**

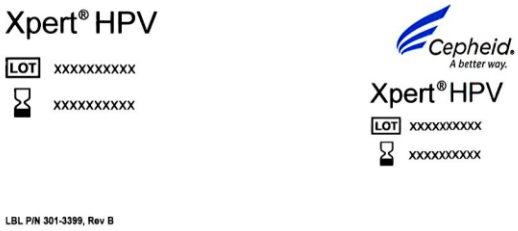
1. Labels

Cartridge label

Box label



Corner label



GeneXpert
Powered By CEPHEID INNOVATION

Xpert® HPV

REF **GXHPV-CE-10**

Cepheid

In Vitro Medical Device IVD CE

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Cepheid AB
Röntgenvägen 5
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Sweden

Xpert® HPV

For *in vitro* diagnostic use only.

1 Proprietary Name

Xpert® HPV

2 Common or Usual Name

Xpert HPV Assay

3 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 high risk Human Papillomavirus (HPV) in patient specimens. The test carries out multiplexed amplification of target DNA by real-time Polymerase Chain Reaction (PCR) of 14 high risk HPV types in a single analysis. Xpert HPV specifically identifies types HPV 16 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. Specimens are limited to cervical cells collected in PreservCyt® Solution (Hologic Corp.). Cervical specimens collected in PreservCyt Solution that have been pretreated with Glacial Acetic Acid (GAA) to lyse excess red blood cells for cytology review have also been validated for use with the Xpert HPV Assay.

Indications for the Xpert HPV Assay:

- The Xpert HPV Assay can be used with a Pap specimen to assess the presence or absence of high risk HPV types. This information, together with the physician's assessment of the patient's medical history, other risk factors, and professional guidelines, may be used to guide patient management.
- The Xpert HPV Assay can be used with a Pap specimen to assess the presence or absence of HPV genotypes 16 and 18/45. This information, together with the physician's assessment of the patient's medical history, other risk factors, and professional guidelines, may be used to guide patient management.

4 Summary and Explanation

Persistent infection with high risk HPV is the main cause of cervical cancer and is a precursor to cervical intraepithelial neoplasia (CIN). HPV presence has been implicated in more than 99% of cervical cancers worldwide.[1] HPV is a small, non-enveloped, double-stranded DNA virus, with a genome of approximately 8,000 nucleotides. There are more than 150 different types of HPV, and approximately 40 types of HPV that can infect the human anogenital mucosa.[2] However, only a subset of approximately 14 of these types is considered high risk for the development of cervical cancer and its precursor lesions. Recent findings suggest that type-specific high risk HPV-DNA-based screening tests and protocols should focus on HPV types 16, 18, and 45.[3] On a global basis, HPV types 16, 18, and 45 were found in 75% of all squamous carcinomas, and determined to be associated with approximately 80% of all invasive cervical cancers.[4,5]

Note In this publication "HPV" or "HR HPV" means "high risk HPV," unless noted otherwise.

5 Principle of the Procedure

The Xpert HPV Assay is an automated test for qualitative detection and differentiation of HPV DNA. The assay is performed on Cepheid GeneXpert Instrument Systems.

GeneXpert Instrument Systems automate and integrate sample processing, cell lysis, purification, nucleic acid amplification, and detection of the target sequences in clinical samples by using real-time PCR. The systems consist of an instrument, personal computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable GeneXpert cartridges that hold the PCR reagents, house the sample, and carry out the PCR processing. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, refer to the appropriate *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

The Xpert HPV Assay includes reagents for the detection of high risk HPV. The Xpert HPV Assay is designed for use with cervical specimens collected in PreservCyt with either a broom-like device or an endocervical brush/spatula combination. Cervical specimens pretreated with certain Glacial Acetic Acid (GAA) methods may also be used. Cervical specimens collected in PreservCyt Solution have been validated for use with the Xpert HPV Test. Follow the manufacturer's instructions for collecting cervical specimens.

A Sample Adequacy Control (SAC) and a Probe Check Control (PCC) are also included in the cartridge. SAC reagents detect the presence of a single copy human gene and monitor whether the specimen contains adequate numbers of human cells to carry out a qualitative assessment of HPV status. The PCC verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The six color channels contain primers and probes for the detection of specific genotypes or pooled results as follows: “SAC; Primary” for the Sample Adequacy Control, “HPV 16; Primary” for HPV 16, “HPV 18_45; Primary” for the HPV 18/45 pooled result, “P3; Primary” for the pooled result of any of HPV types 31, 33, 35 52, or 58, “P4: Primary” for the pooled result of either of HPV types 51 or 59, and “P5; Primary” for the pooled result of any of HPV types 39, 56, 66 or 68. For an example of the assay legend, see Figure 5.

6 Reagents and Instruments

6.1 Material Provided

The Xpert HPV Assay kit (GXHPV-CE-10) contains sufficient reagents to process 10 quality control samples and/or specimens.

The kit contains the following:

Xpert HPV Assay cartridges with integrated reaction tubes	**10**
• Bead 1 and 2 (freeze-dried)	1 of each per cartridge
• Buffer Reagent	2.0 mL per cartridge
Transfer pipettes (1 mL)	**10**
CD	**1**
• Assay Definition Files (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 postmortem testing. During processing, there was no mixing of the material with other animal materials.

6.2 Storage and Handling

- Store the Xpert HPV Assay cartridges and reagents at 2–28 °C.
- Do not open a cartridge until ready to test. Use cartridges within 30 minutes after opening the cartridge lid.

- Do not use reagents or cartridges that have passed the expiration date.
- Do not use a cartridge that has leaked.

6.3 Materials Required but Not Provided

- Cervical specimen collected in PreservCyt with either a broom-like device or an endocervical brush/spatula combination
- GeneXpert Dx instrument (catalog number varies by configuration): six-color GeneXpert instrument, computer with proprietary GeneXpert Software Version 4.3 or higher, and barcode scanner

  or

  GeneXpert Infinity system with Xpertise software version 6.1 or higher
- Appropriate GeneXpert Instrument System operator manual
- Printer (If a printer is required, contact Cepheid Technical Support to arrange for the purchase of a recommended printer.)

7 Warnings and Precautions

7.1 General

- For *in vitro* diagnostic use.
- Pathogenic microorganisms, including hepatitis viruses and human immunodeficiency virus (HIV), may be present in clinical samples. Treat all biological samples, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological samples should be treated with standard precautions. Guidelines for sample handling are available from the U.S. Center for Disease Control and Prevention and the Clinical and Laboratory Standards Institute.[6,7]
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- 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.
- Good laboratory practices and changing gloves between handling patient specimens are recommended to avoid contamination of specimens.

7.2 Specimen Collection, Transport, and Storage

- **Specimen Collection**

  Cervical specimens collected in PreservCyt Solution have been validated for use with the Xpert HPV Assay. Follow the manufacturer's instructions for collecting cervical specimens.

- **Specimen Transport**

  Cervical specimens collected in PreservCyt Solution can be transported at 2–30 ºC. Transportation of HPV specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.[8]

- **Specimen Storage**

  Cervical specimens collected in PreservCyt Solution may be stored at 2–30 °C for up to six months after the date of collection.

7.3 Assay/Reagent

- Do not substitute Xpert HPV Assay reagents with other reagents.
- Do not open the Xpert HPV Assay cartridge lid until you are ready to add a sample during testing.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge may yield invalid results.
- Do not place the sample ID label on the cartridge lid or on the bar code label.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use Xpert HPV Assay cartridge is used to process one test. Do not reuse processed cartridges.
- Wear clean lab coats and gloves. Change gloves between processing each sample.
- In the event of contamination of the work area or equipment with samples or controls, thoroughly clean the contaminated area with a concentration of 1:10 dilution of household chlorine bleach andthen a 70% ethanol or 70% isopropanol solution. Wipe work surfaces dry completely before proceeding.

8 Chemical Hazards[9,10]

Ingredients are not considered hazardous under EU directives for classification and labeling of substances or mixtures or the Global Harmonization System for classification and labeling of substances or mixtures.

9 Procedure

Before starting these procedures, make sure that the GeneXpert instrument contains six-color modules with GX Dx software version 4.3 or higher or Xpertise software version 6.1 or higher.

Important **Start the test within 30 minutes of opening the cartridge lid.**

9.1 Preparing the Cartridge

To add the sample to the Xpert HPV Assay cartridge:

1. Obtain the following items:
   - Xpert HPV Assay cartridge.
   - Transfer pipette (provided). Line on pipette indicates 1 mL fill volume.
   - Appropriately collected and labeled test sample.
2. Inspect the test cartridge for damage. If damaged, do not use it.
3. Open the cartridge lid.
4. Mix the sample by gently inverting the sample vial 8 to 10 times, or by vortexing briefly with a vortex mixer at half-speed continuously for 5 seconds.
5. Unwrap the transfer pipette.
6. Open the sample vial lid, compress the transfer pipette bulb, insert the pipette into the vial, and release the bulb to fill the transfer pipette to the 1 mL line. See Figure 1. Ensure the pipette is filled, with no air bubbles present.

Important **Avoid adding excess mucus to the cartridge.**

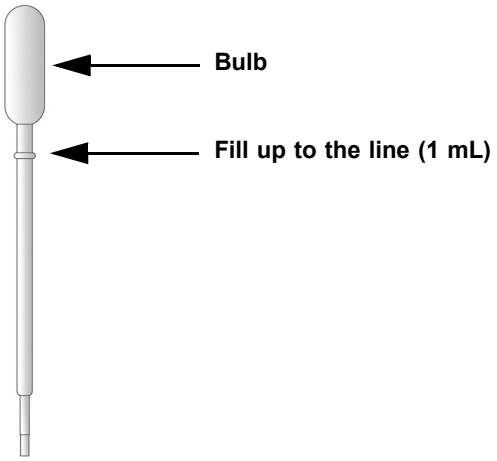

Figure 1. Transfer Pipette and Fill Mark

7. Expel the pipette's contents into the sample chamber of the cartridge. See Figure 2.

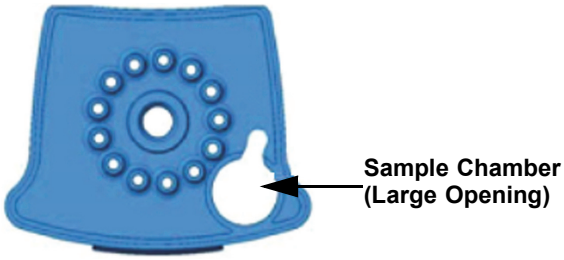

Figure 2. Xpert HPV Assay Cartridge (Top View)

8. Close the cartridge lid.

9.2 Starting the Test

Important **Before you start the test, make sure the Xpert HPV Assay Definition Files (ADF) are imported into the software. This section lists the basic steps of running the test. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual.***

Note The steps you follow can be different if the system administrator changed the default workflow of the system.

This section lists the default steps to operate the GeneXpert System. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the model that is being used.

1. Turn on the GeneXpert Instrument System:
   - If using the GeneXpert Dx instrument, first turn on the instrument and then turn on the computer. The GeneXpert software will launch automatically or may require double-clicking the GeneXpert Dx software shortcut icon on the Windows® desktop.

     or

   - If using the GeneXpert Infinity instrument, power up the instrument. The GeneXpert software will launch automatically or may require double clicking the Xpertise software shortcut icon on the Windows desktop.
2. Log on to the GeneXpert Instrument System software using your user name and password.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or click **Orders** and **Order Test** (Infinity). The Create Test window appears. See Figure 3.

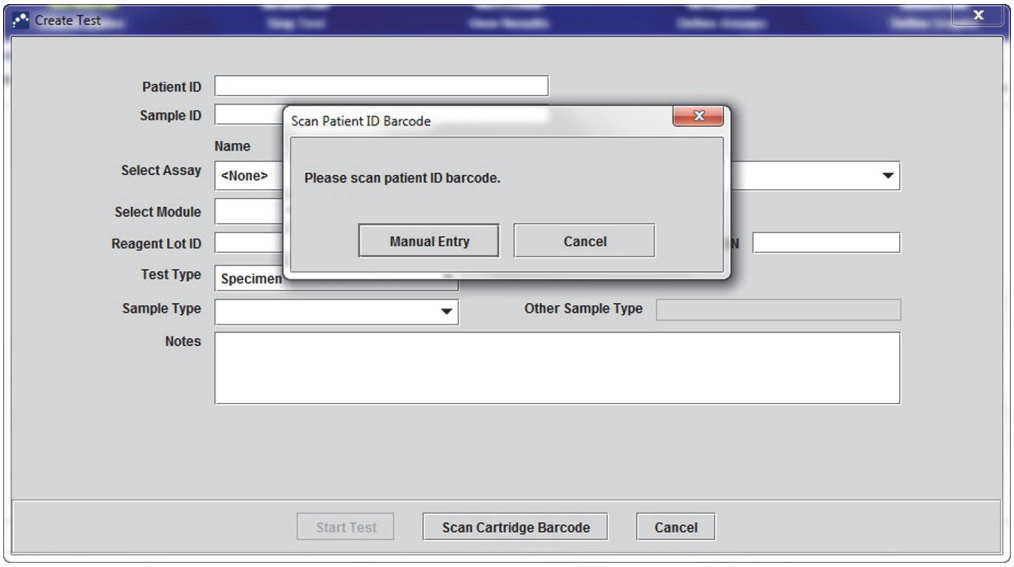

Figure 3. GeneXpert Dx Create Test Window

4. Scan or type in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is associated with the test results and is shown in the View Results window.
5. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is associated with the test results and is shown in the View Results window and all reports. The Scan Cartridge dialog box appears.
6. Scan the barcode on the Xpert HPV cartridge. The Create Test window appears. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

Note If the barcode on the Xpert HPV Assay cartridge does not scan, then repeat the test with a new cartridge following the procedure in Section 14, Retest Procedure.

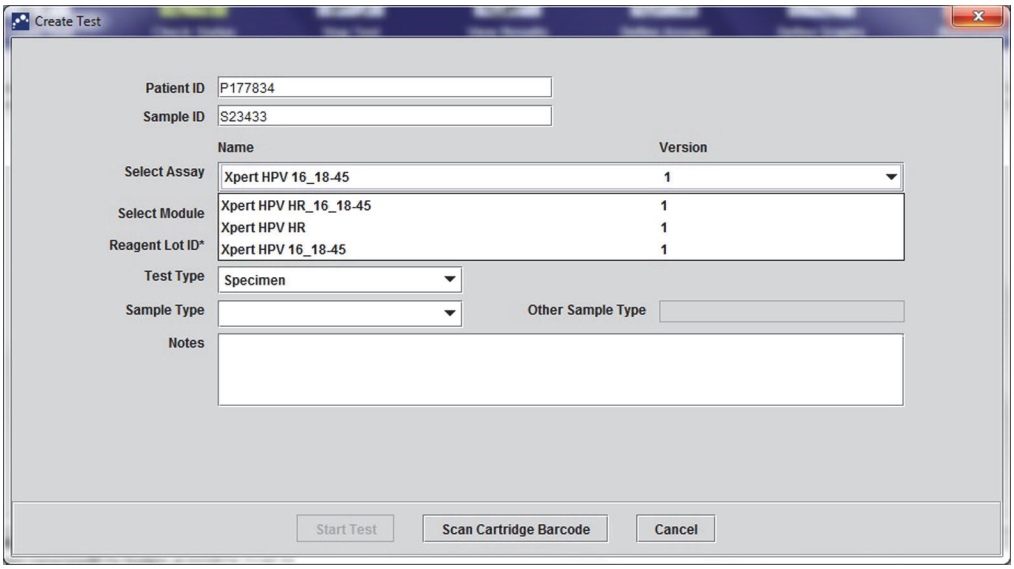

Figure 4. GeneXpert Dx Create Test Window with Select Assay Drop-down Menu

7. From the **Select Assay** drop-down menu (see Figure 4), select the appropriate Assay Definition File (ADF) for the HPV test ordered.

   The Xpert HPV Assay can be configured to default to any one of the three ADFs at the discretion of the laboratory. Clinician requests for reflex genotyping of HPV 16 or HPV 18/45, can be ordered under the HPV genotype specific assay, or where indicated, run as part of a full high risk and genotype assay.

   - High risk HPV only test: Selecting assay **Xpert HPV HR** reports a positive or a negative overall result for the presence of any of the 14 high risk HPV types detected. An example is shown in Figure 5.
   - HPV 16, 18/45 genotyping test: Selecting assay **Xpert HPV 16_18-45** reports a positive or a negative result for:
     - HPV 16, and for
     - HPV 18 or HPV 45 genotype.

     Specific results of all other HPV types are neither collected nor displayed. An example is shown in Figure 6.
   - A combined high risk HPV and HPV genotype test: Selecting assay **Xpert HPV HR_16_18-45** reports a positive or a negative result for HPV 16, for HPV 18/45, and for the presence of any of the remaining 11 other high risk types as "Other HR HPV." An example is shown in Figure 7.

Note Only the test result for the assay selected at this step will be collected once the test is started. Uncollected data are not recoverable.

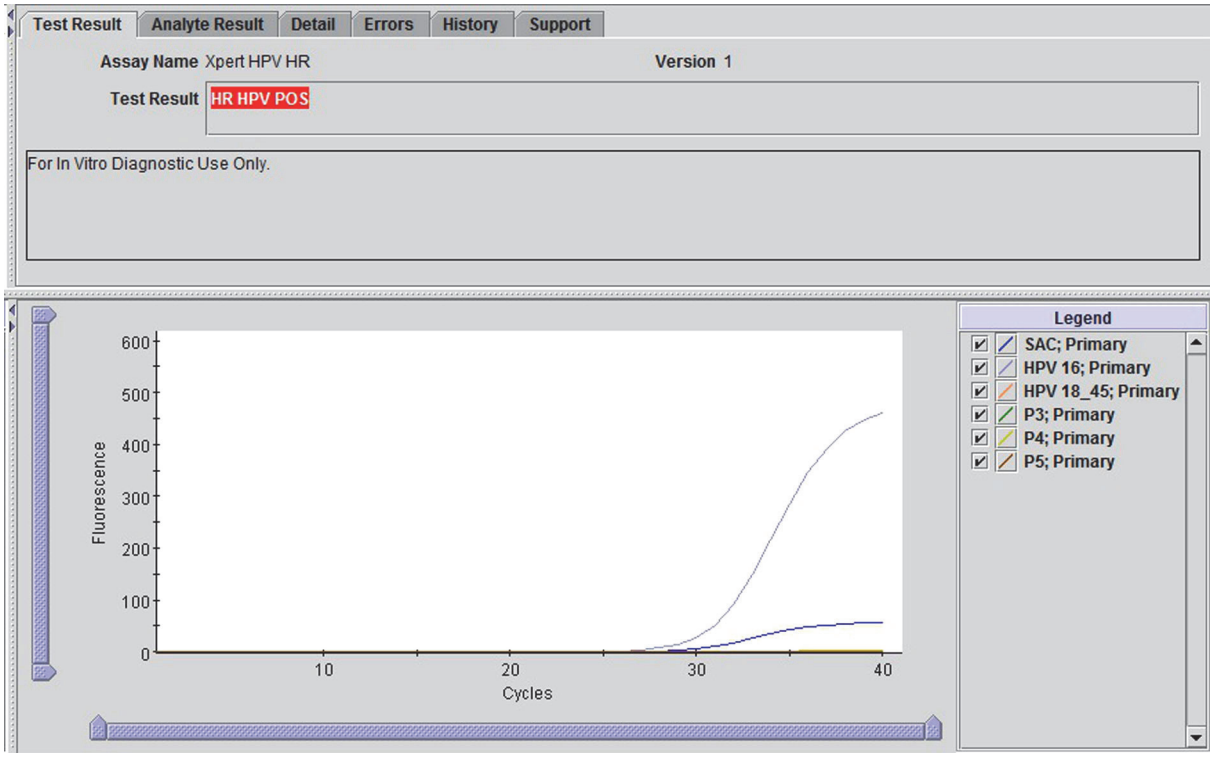

Figure 5. HPV HR Positive

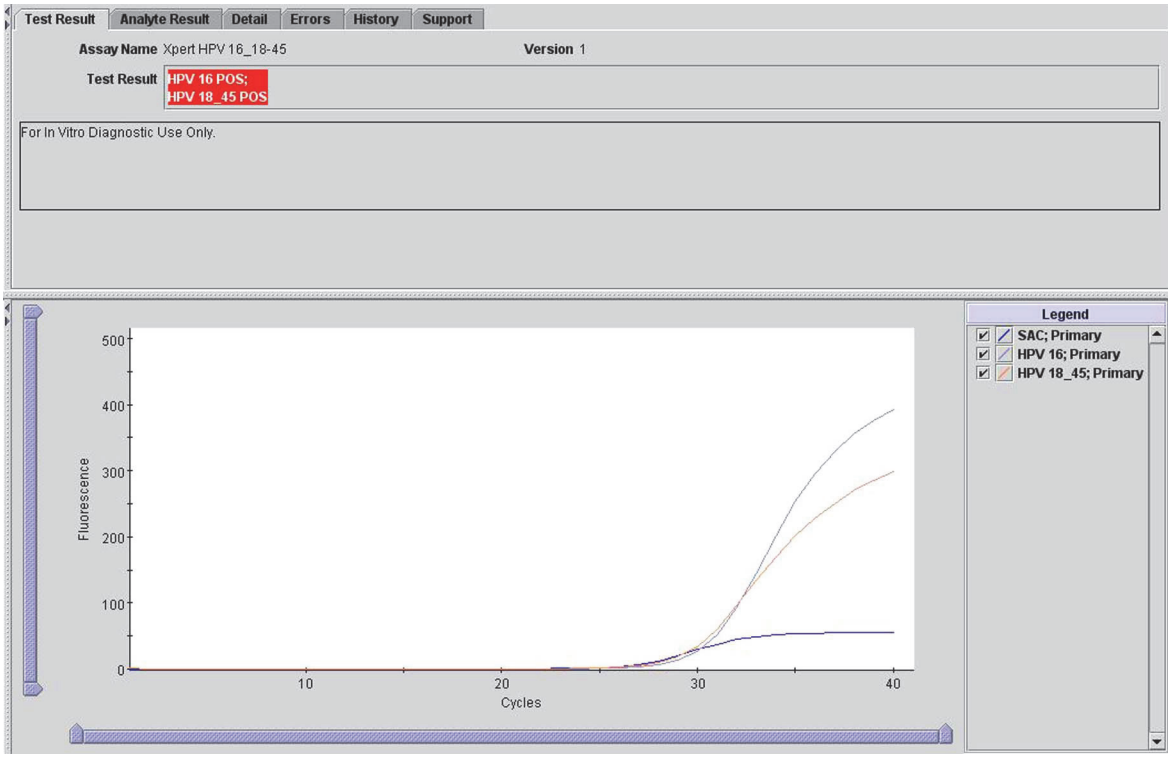

Figure 6. HPV 16_18-45 Positive

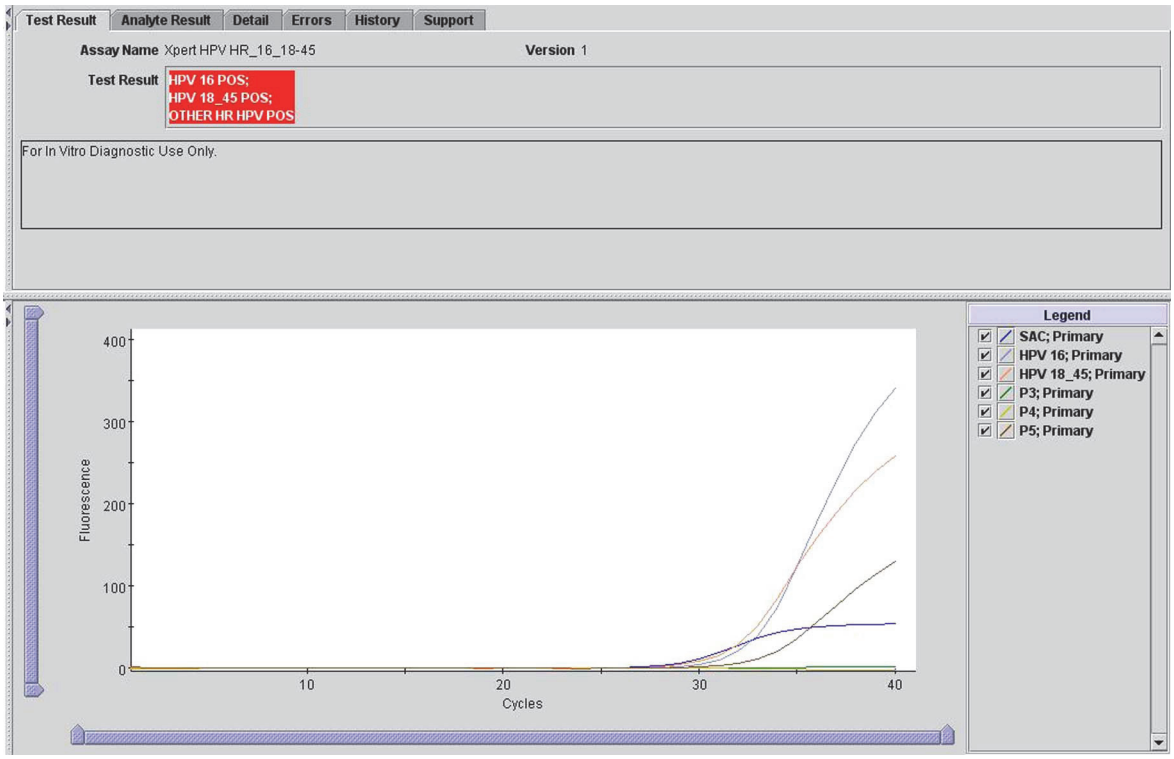

Figure 7. HPV HR_16_18-45 Positive

8. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity). Enter your password, if requested.
9. For the GeneXpert Infinity System, place the cartridge on the conveyor belt. The cartridge will be automatically loaded, the test will run, and the used cartridge will be placed into the waste container.

   or

   For the GeneXpert Dx Instrument:

   A. Open the instrument module door with the blinking green light and load the cartridge.
   B. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
   C. Wait until the system releases the door lock before opening the module door and removing the cartridge.
   D. The used cartridges should be disposed in the appropriate specimen waste containers according to your institution's standard practices.

Note Time to result is approximately 60 minutes.

10 Viewing and Printing Results

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

11 Quality Control

CONTROL

Each test includes a Probe Check Control (PCC) and a Sample Adequacy Control (SAC).

- **Probe Check Control (PCC):** Before the PCR reaction starts, the GeneXpert instrument measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity and dye stability. PCC passes if it meets the validated acceptance criteria.
- **Sample Adequacy Control (SAC):** The SAC reagents detect the presence of a single copy human gene present in one copy per cell and monitor whether the sample contains human DNA.
- **External Controls:** External controls may be used in accordance with local, state, federal accrediting organizations, as applicable.

12 Interpretation of Results

The results are interpreted by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and will be shown on the Test Result tab of the View Results window. The Xpert HPV Assay provides test results for HPV targets, according to the results and interpretations shown in Table 1.

Note Only the test results for the selected assay will be collected once the test is started.

Table 1. Xpert HPV Assay Results and Interpretations

Result	Interpretation
HR HPV POS See Figure 9.	High risk HPV DNA is detected as positive. • The targeted high risk HPV DNA has a Ct within the valid range and a fluorescence endpoint above the threshold setting. • SAC: Not applicable. The SAC is ignored because HPV target amplification can compete with this control. • PCC: PASS; all probe check results pass.
HPV 16 POS See Figure 11, Figure 13, and Figure 16.	HPV 16 DNA is detected as positive. • The targeted HPV 16 DNA has a Ct within the valid range and a fluorescence endpoint above the threshold setting. • SAC: Not applicable. The SAC is ignored because HPV target amplification can compete with this control. • PCC: PASS; all probe check results pass.
HPV 18_45 POS See Figure 14 and Figure 16.	HPV 18_45 DNA is detected as positive. • The targeted HPV 18/45 DNA has a Ct within the valid range and a fluorescence endpoint above the threshold setting. • SAC: Not applicable. The SAC is ignored because HPV target amplification can compete with this control. • PCC: PASS; all probe check results pass.
OTHER HR HPV POS See Figure 15 and Figure 16.	Other high risk HPV DNA is detected as positive. • The targeted other high risk HPV DNA has a Ct within the valid range and a fluorescence endpoint above the threshold setting. • SAC: Not applicable. The SAC is ignored because other high risk HPV target amplification can compete with this control. • PCC: PASS; all probe check results pass.
HR HPV NEG See Figure 8.	High risk HPV DNA is below the level of detection. • The targeted high risk HPV DNA has a Ct not within the valid range and/or a fluorescence endpoint below the threshold setting. • SAC: PASS; PCR amplification of the SAC target gives a Ct within the valid range and a fluorescence endpoint above the threshold setting. • PCC: PASS; all probe check results pass.

Table 1. Xpert HPV Assay Results and Interpretations (Continued)

Result	Interpretation
HPV 16 NEG See Figure 10, Figure 12, Figure 14, and Figure 15.	HPV 16 DNA is below the level of detection. • The targeted HPV 16 DNA has a Ct not within the valid range and/or a fluorescence endpoint below the threshold setting. • SAC: PASS; PCR amplification of the SAC target gives a Ct within the valid range and a fluorescence endpoint above the threshold setting. • PCC: PASS; all probe check results pass.
HPV 18_45 NEG See Figure 10, Figure 11, Figure 12, Figure 13, and Figure 15.	HPV 18-45 DNA is below the level of detection. • The targeted HPV 18/45 DNA has a Ct not within the valid range and/or a fluorescence endpoint below the threshold setting. • SAC: PASS; PCR amplification of the SAC target gives a Ct within the valid range and a fluorescence endpoint above the threshold setting. • PCC: PASS; all probe check results pass.
OTHER HR HPV NEG See Figure 12, Figure 13, and Figure 14.	Other high risk HPV DNA is below the level of detection. • The targeted other high risk HPV DNA has a Ct not within the valid range and/or a fluorescence endpoint below the threshold setting. • SAC: PASS; PCR amplification of the SAC target gives a Ct within the valid range and a fluorescence endpoint above the threshold setting. • PCC: PASS; all probe check results pass.
INVALID See Figure 17.	Presence or absence of HPV target DNA cannot be determined. Repeat the test according to the instructions in Section 14, Retest Procedure. • SAC: FAIL; SAC Ct is not within the valid range and/or a fluorescence endpoint below the threshold setting. • PCC: PASS; all probe check results pass.
ERROR	Presence or absence of HPV target DNA cannot be determined. Repeat the test according to the instructions in Section 14, Retest Procedure. • SAC: NO RESULT • PCC: FAIL*; all or one of the probe check results fail. * If the probe check passed, the error is caused by the maximum pressure limit exceeding the acceptable range or by a system component failure.
NO RESULT	Presence or absence of HPV target DNA cannot be determined. Repeat the test according to the instructions in Section 14, Retest Procedure. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress or a power failure occurred. • HPV: NO RESULT • SAC: NO RESULT • PCC: NA (not applicable)

Note The screens shown in this section reflect examples using the three assays. Figure 8 and Figure 9 use the Xpert HPV HR assay, Figure 10 and Figure 11 use the Xpert HPV 16_18-45 genotype assay, and Figure 12 through Figure 14 use the Xpert HPV HR_16_18-45 assay from the drop down menu. (See Section 9.2, Starting the Test and the drop-down menu illustrated in Figure 4).

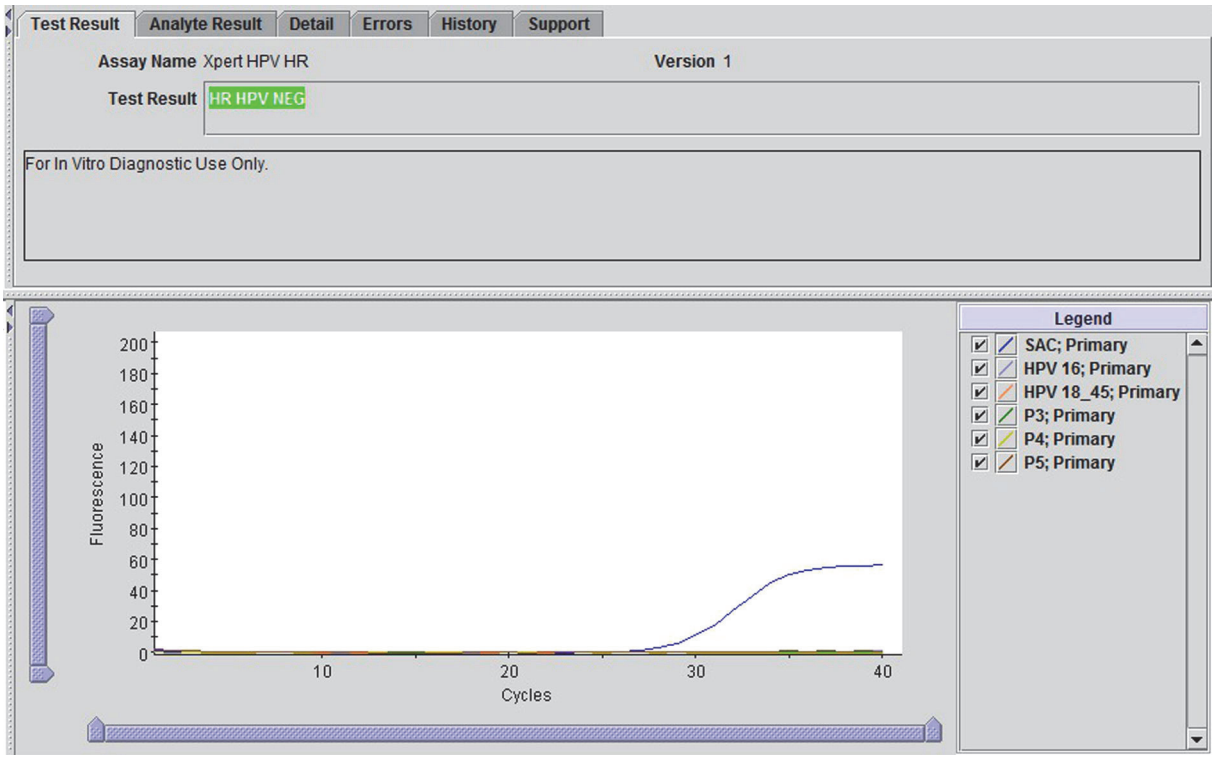

Figure 8. High Risk HPV Negative (Result Using Xpert HPV HR Assay)

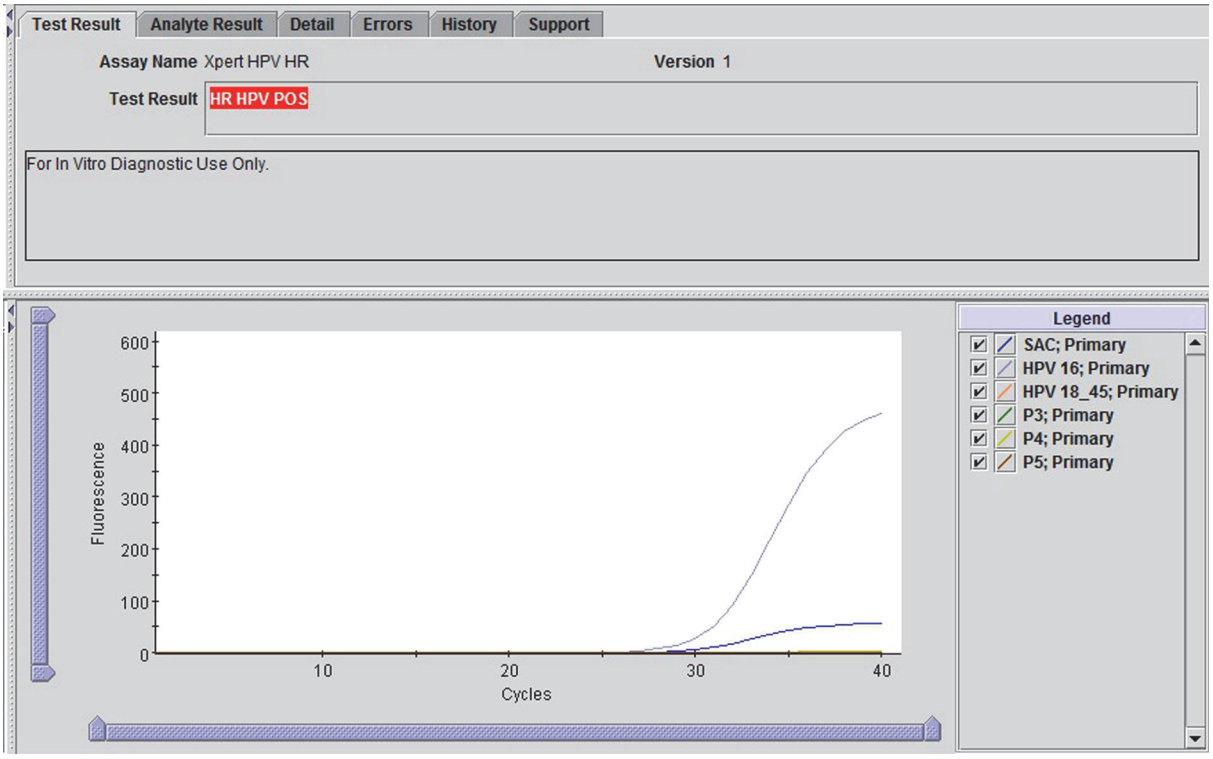

Figure 9. High Risk HPV Positive (Result Using Xpert HPV HR Assay)

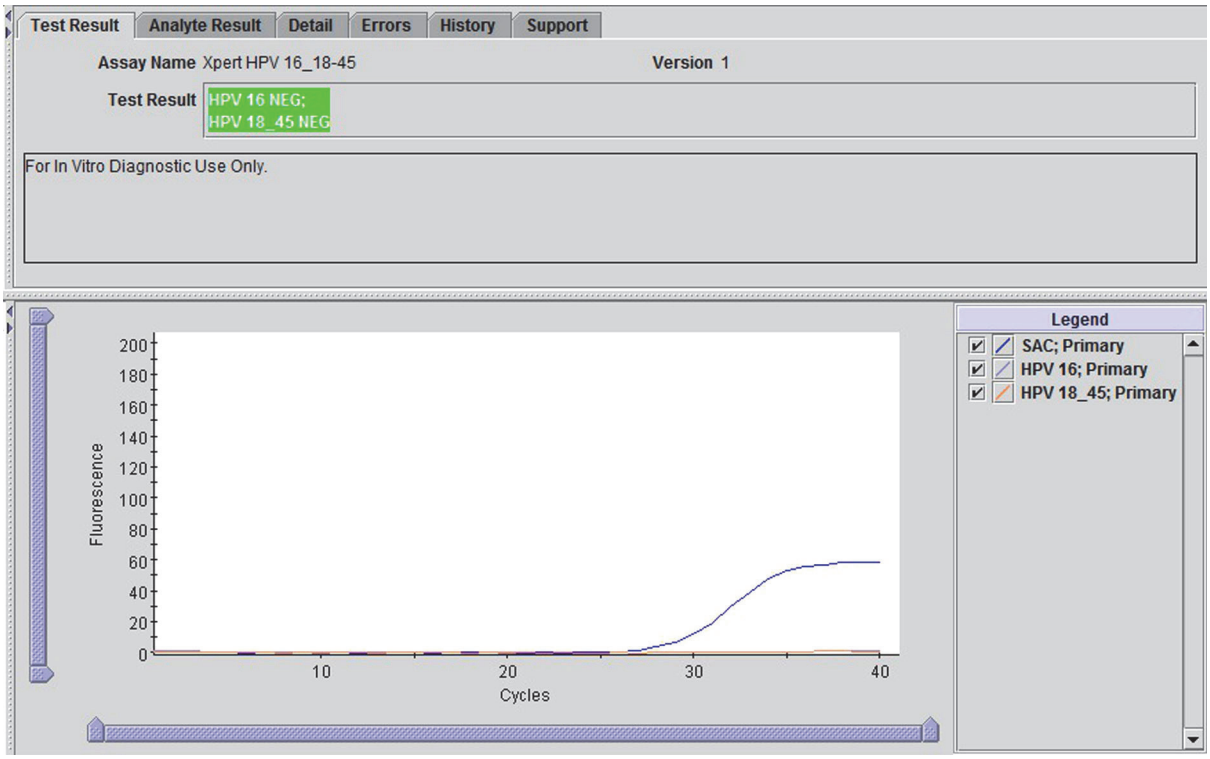

Figure 10. HPV 16 Negative; HPV 18-45 Negative (Result Using Xpert HPV 16_18-45 Assay)

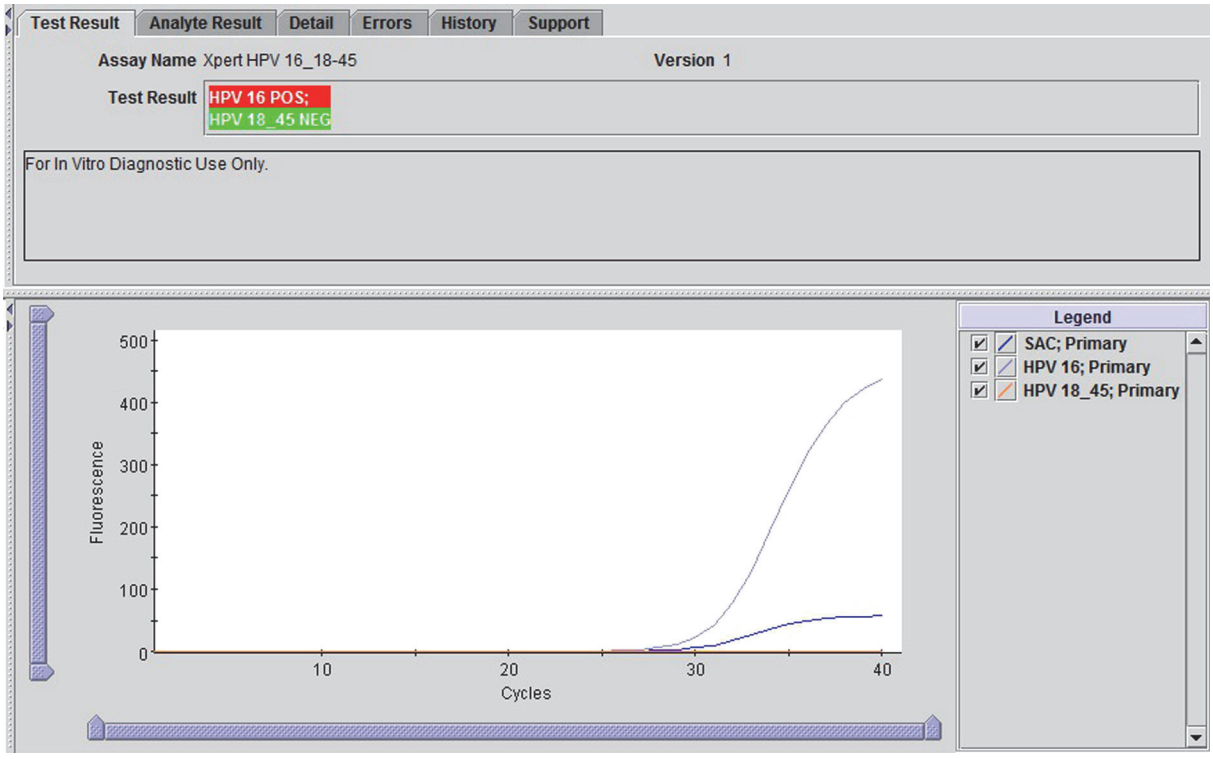

Figure 11. HPV 16 Positive; HPV 18-45 Negative (Result Using Xpert HPV 16_18-45 Assay)

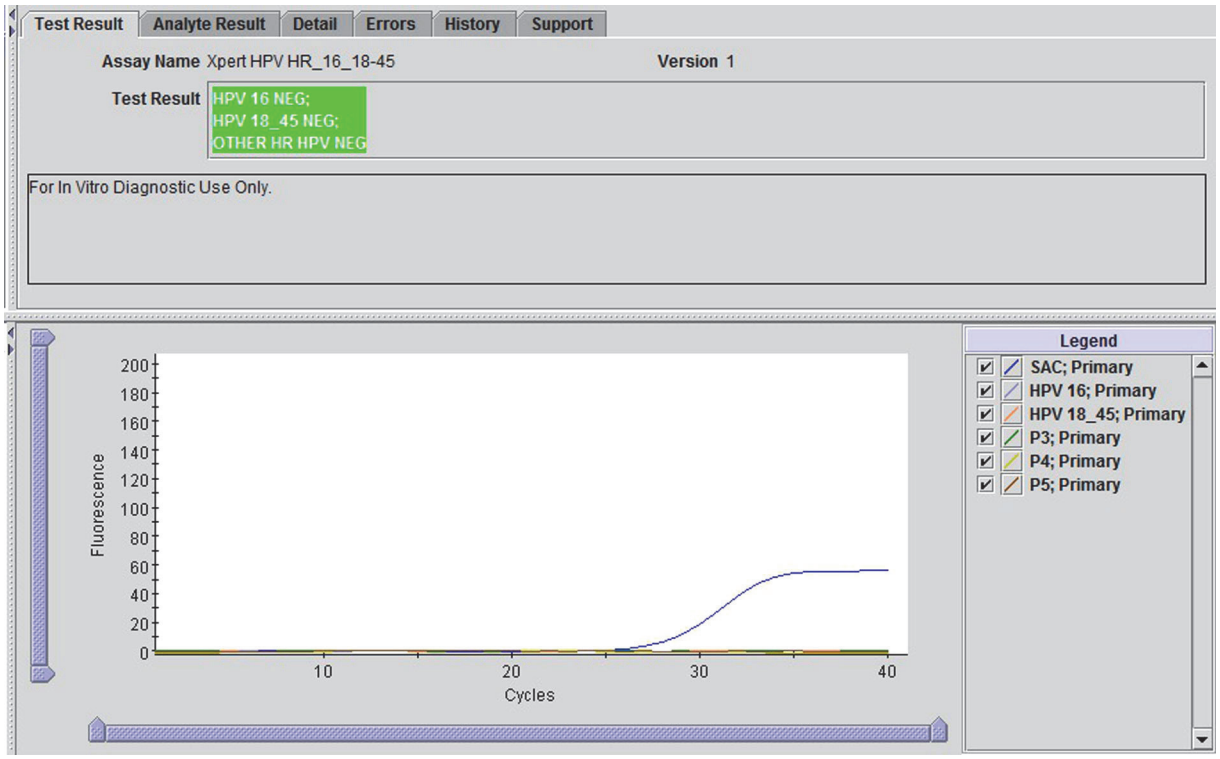

Figure 12. HPV 16 Negative; HPV 18-45 Negative; Other High Risk HPV Negative (Result Using Xpert HPV HR_16_18-45 Assay)

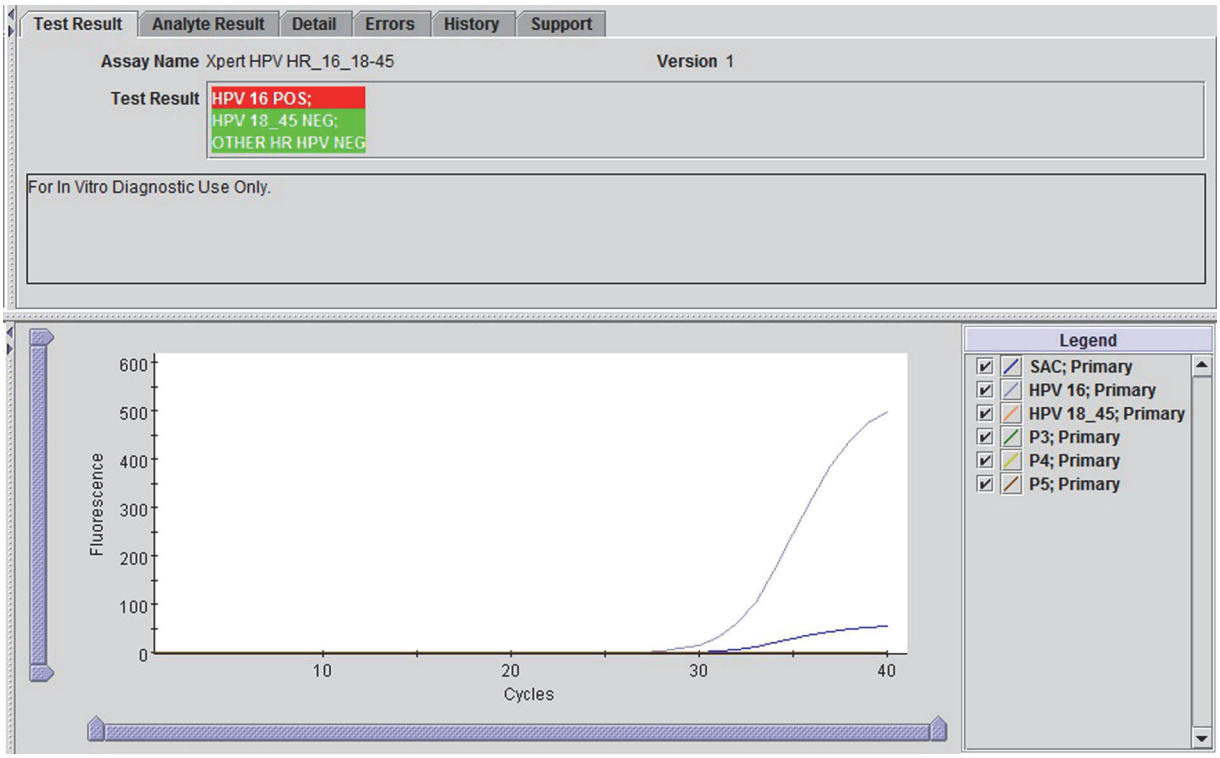

Figure 13. HPV 16 Positive; HPV 18-45 Negative; Other High Risk HPV Negative (Result Using Xpert HPV HR_16_18-45 Assay)

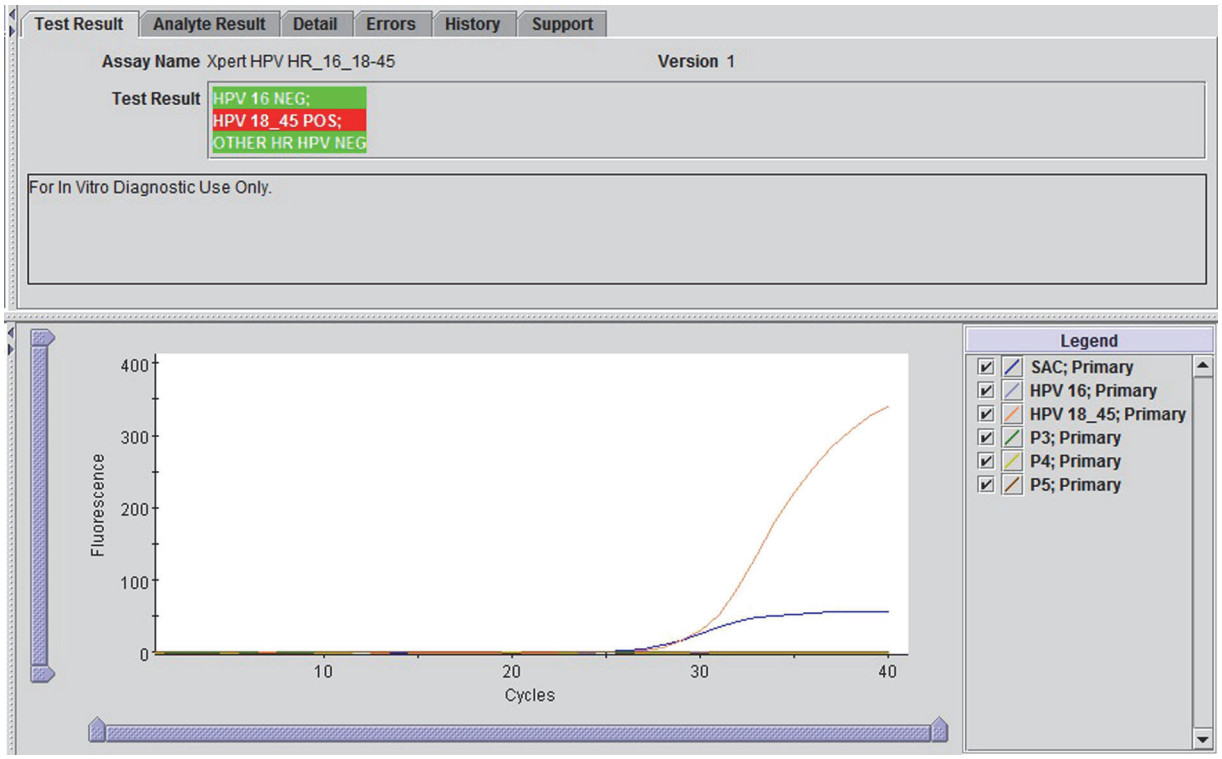

Figure 14. HPV 16 Negative; HPV 18-45 Positive; Other High Risk HPV Negative (Result Using Xpert HPV HR_16_18-45 Assay)

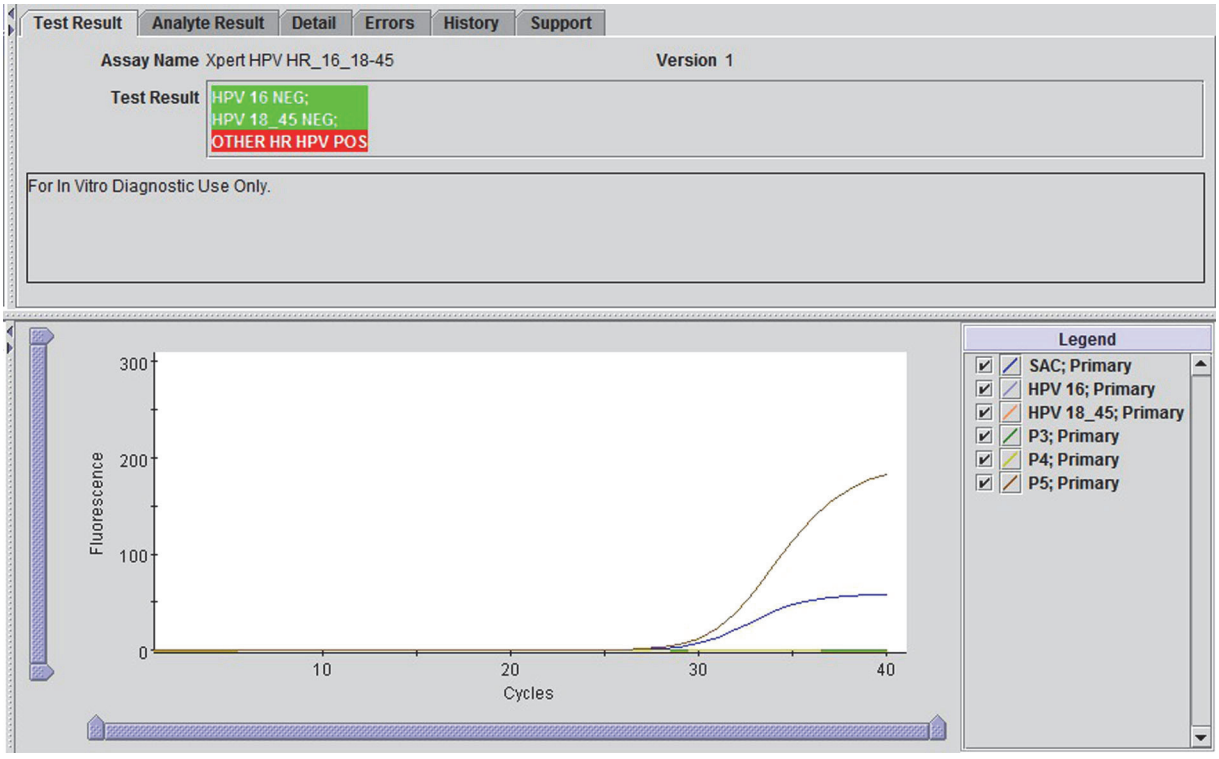

Figure 15. HPV 16 Negative; HPV 18-45 Negative; Other High Risk HPV Positive (Result Using Xpert HPV HR_16_18-45 Assay)

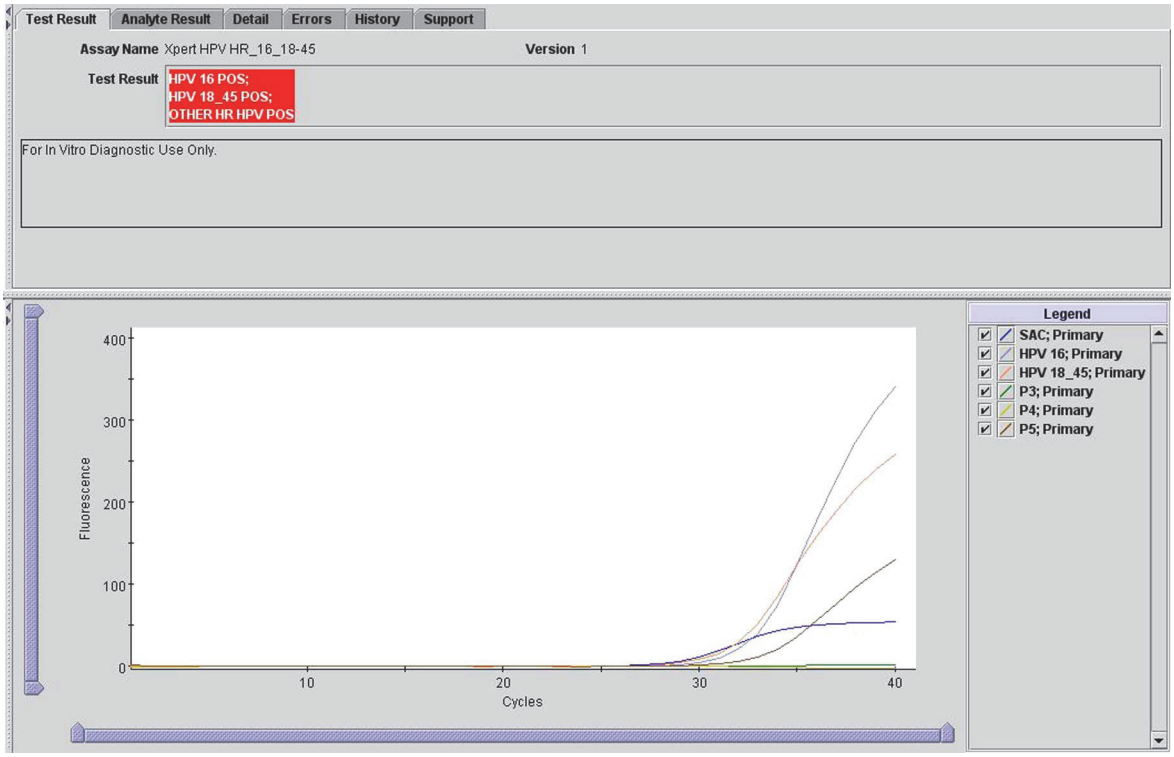

Figure 16. HPV 16 Positive, HPV18-45 Positive; Other High Risk HPV Positive (Result Using Xpert HPV HR_16_18-45 Assay)

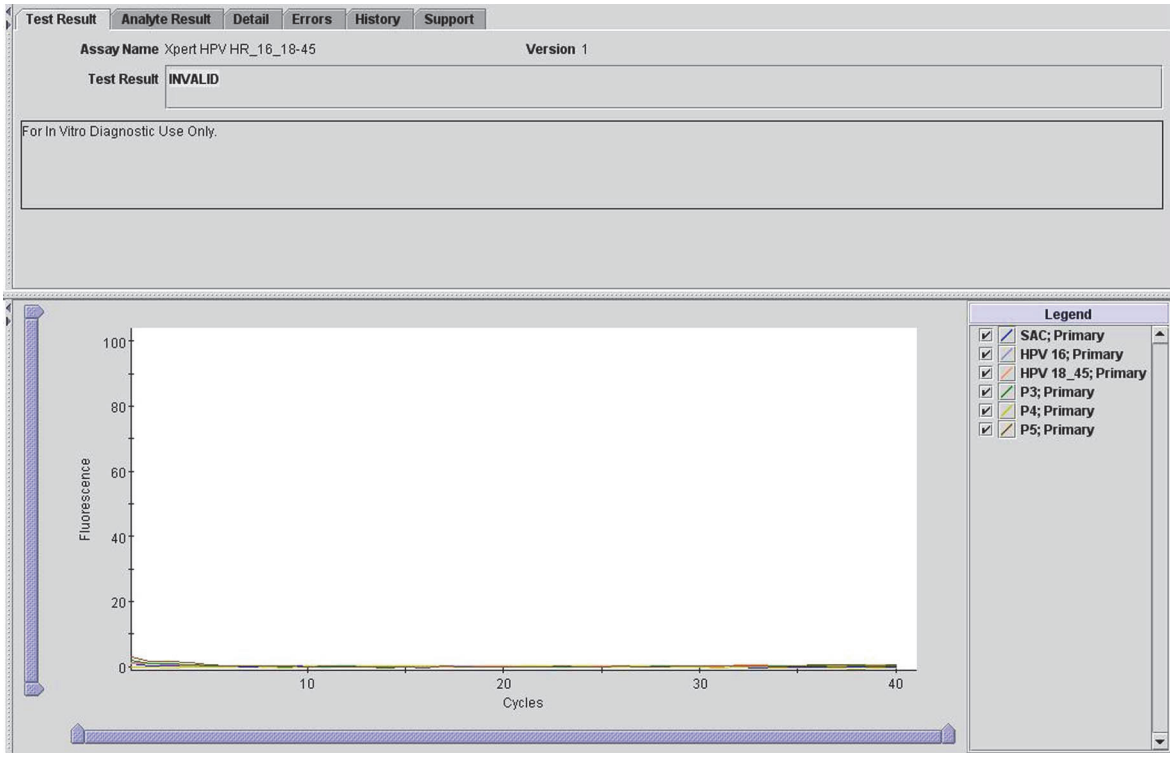

Figure 17. HPV HR_16_18-45 Invalid (Result Using Xpert HPV HR_16_18-45 Assay)

13 Reasons to Repeat the Assay

If any of the following test results occur, repeat the test according to instructions in Section 14, Retest Procedure.

- An **INVALID** result indicates that the SAC failed, the sample was not properly processed, PCR was inhibited, or the sample was inadequate.
- An **ERROR** result indicates that the test was aborted, possibly because the reaction tube was filled improperly, a reagent probe integrity problem was detected, pressure limits were exceeded, a probe check failed, or a valve positioning error was detected.
- A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress or a power failure occurred.

14 Retest Procedure

- Repeat the test with a new cartridge (do not re-use the cartridge). See Section 9, Procedure.
- 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 and repeat the test with a new cartridge.

15 Limitations

- Because the detection of HPV is dependent on the DNA present in the sample, reliable results are dependent on proper sample collection, handling, and storage.
- 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.
- Erroneous test results might occur from improper specimen collection, technical error, sample mix-up, or because the HPV DNA copy number is below the limit of detection of the test.
- The Xpert HPV Assay has been validated using the procedures provided in this package insert only. Modification to these procedures may alter the performance of the test.
- Assay interference may be observed in the presence of: whole blood ($\geq$ 0.25% v/v), peripheral blood mononuclear cells (PBMC) ($\geq 1 \times 10^6$ cells/mL), *Candida albicans* ($\geq 1 \times 10^8$ cells/mL), Vagisil anti-itch cream ($\geq$ 0.25% w/v) or Vagi Gard moisturizing gel ($\geq$ 0.5% w/v).
- The presence of thick vaginal creams ($>$ 0.25% w/v) in the sample may result in pressure aborts.
- The effects of other potential variables such as vaginal discharge, use of tampons, douching, and specimen collection variables have not been determined.
- The Xpert HPV Assay provides qualitative results. No correlation can be drawn between the magnitude of the Ct value and the number of cells in an infected sample.
- Xpert HPV Assay performance has not been evaluated in patients less than 18 years of age.
- Xpert HPV Assay performance has not been evaluated in women with a history of hysterectomy.
- The Xpert HPV Assay has not been validated for use with vaginal swab specimens collected by a physician or a patient.
- The Xpert HPV Assay has not been evaluated with patients who are currently being treated with antimicrobial agents for infections such as chlamydia or gonorrhea.
- As with many diagnostic tests, results from the Xpert HPV Assay should be interpreted in conjunction with other laboratory and clinical data available to the physician.
- The performance of the Xpert HPV Assay has not been evaluated for HPV-vaccinated individuals.
- The Xpert HPV Assay has not been evaluated in cases of suspected sexual abuse.
- Prevalence of HPV infection in a population may affect performance.
- Samples containing less than 1 mL of PreservCyt Solution are considered inadequate for the Xpert HPV Assay.
- Xpert HPV Assay performance has not been evaluated in cervical specimens preprocessed for cytology review using processors other than the ThinPrep 2000 Processor.
- A negative Xpert HPV Assay result does not exclude the possibility of cytologic abnormalities or of future or underlying CIN2, CIN3, or cancer.

- The Xpert HPV Assay detects E6/E7 viral DNA of the high risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. This test does not detect E6/E7 DNA of HPV low risk types (e.g., 6, 11, 42, 43, 44) since there is no clinical utility for assessing the presence of low risk types of HPV in the context of cervical cancer screening.
- Detection of high risk HPV DNA is dependent on the number of copies present in the specimen and may be affected by specimen collection methods, patient factors, stage of infection, and the presence of interfering substances.
- Use of this product must be limited to personnel trained in the use of the Xpert HPV Assay.
- False Positive or False Negative results may occur with this test.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of targeted HPV types resulting in a false negative result.

16 Clinical Performance

Clinical performance characteristics of the Xpert HPV Assay were assessed in a two-stage, multicenter [seven US sites], prospective study that enrolled women of all ages referred for colposcopy evaluation based on one or more prior abnormal Pap test results, an abnormal Pap test result in combination with a positive high risk HPV test result, or other clinical suspicion of cervical cancer. Two ThinPrep specimens (Specimen A and Specimen B) were collected from each subject at the time of colposcopy to support cytology review and comparator testing with the Xpert HPV Assay and two FDA-approved, high risk HPV tests. Analyses with these comparator methods were conducted per the respective US-IVD Package Inserts. Specimen A was processed for cytology review followed by analysis with the Xpert HPV Assay. Specimen B was reserved for HPV analysis with the comparator HPV tests and the Xpert HPV Assay. Both specimens were collected using an endocervical brush/spatula combination per the ThinPrep Package Insert. A minimum of two cervical punch biopsies were collected from each subject as well as an ECC for unsatisfactory colposcopy evaluations in which there was poor visualization of the squamocolumnar junction. Pathology review of the biopsy and endocervical curettage (ECC) specimens was first conducted locally for standard of care/patient management and then retrospectively, in blinded fashion, by a panel of three expert review pathologists to determine a consensus final cervical disease status. Stage I of recruitment included 144 subjects (age range: 20–70 years) with 31 cases ≥ CIN2. Data from Stage I was used to estimate a set of clinical cutoffs for the assay relative to ≥ CIN2 and ≥ CIN3 disease end points using a Receiver Operating Characteristic (ROC) approach. Stage II of recruitment included 564 subjects (age range: 18–75 years) with 111 cases ≥ CIN2. Data from Stage II was used to refine the clinical cutoffs relative to ≥ CIN2 and ≥ CIN3 disease end points using an ROC approach. Retrospectively, a homogeneity analysis was conducted to confirm the poolability of results from Stage I and Stage II; across multiple population and specimen parameters, the results are poolable.

Clinical sensitivity and specificity of the Xpert HPV Assay, comparator method 1, and comparator method 2 in the Stage II data set relative to a ≥ CIN2 disease status, are summarized in Table 2.

Table 2. Clinical Performance Relative to ≥ CIN2 Disease Status[a]

	Xpert HPV Assay (Specimen A)[b]	Xpert HPV Assay (Specimen B)[c]	Comparator Method 1[d]	Comparator Method 2[e]
Sensitivity	(99/109) 90.8% (83.8 – 95.5%)	(100/110) 90.9% (83.9 – 95.6%)	(103/111) 92.8% (86.3 – 96.8%)	(96/111) 86.5% (78.7 – 92.2%)
Specificity	(182/429) 42.4% (37.7 – 47.3%)	(194/446) 43.5% (38.8 – 48.2%)	(178/453) 39.3% (34.8 – 44.0%)	(212/451) 47.0% (42.3 – 51.7%)
Positive Predictive Value	(99/346) 28.6% (23.8 – 33.7%)	(100/352) 28.4% (23.8 – 33.4%)	(103/378) 27.2% (22.8 – 32.0%)	(96/335) 28.7% (23.9 – 33.8%)
Negative Predictive Value	(182/192) 94.8% (90.6 – 97.5%)	(194/204) 95.1% (91.2 – 97.6%)	(178/186) 95.7% (91.7 – 98.1%)	(212/227) 93.4% (89.3 – 96.3%)

a. Point estimates are as indicated. Confidence intervals are Fisher-Exact 95% CI.
b. n = 538. Nine specimens QNS for Xpert testing; 17 specimens indeterminate upon initial and retest.
c. n = 556. Eight specimens indeterminate upon initial and retest.
d. n = 564.
e. n = 562. Two specimens indeterminate upon initial and retest.

Clinical sensitivity and specificity of the Xpert HPV Assay, comparator method 1, and comparator method 2 in the Stage II data set relative to a ≥ CIN3 disease status are summarized in Table 3.

Table 3. Clinical Performance Relative to ≥ CIN3 Disease Status[a]

	Xpert HPV Assay (Specimen A)[b]	Xpert HPV Assay (Specimen B)[c]	Comparator Method 1[d]	Comparator Method 2[e]
Sensitivity	(68/72) 94.4% (86.4 – 98.5%)	(69/73) 94.5% (86.6 – 98.5%)	(71/74) 95.9% (88.6 – 99.2%)	(64/74) 86.5% (76.5 – 93.3%)
Specificity	(187/465) 40.2% (35.7 – 44.8%)	(199/482) 41.3% (39.6 – 45.8%)	(182/489) 37.2% (32.9 – 41.7%)	(216/487) 44.4% (39.9 – 48.9%)
Positive Predictive Value	(68/346) 19.7% (15.6 – 24.2%)	(69/352) 19.6% (15.6 – 24.1%)	(71/378) 18.8% (15.0 – 23.1%)	(64/335) 19.1% (15.0 – 23.7%)
Negative Predictive Value	(187/191) 97.9% (94.7 – 99.4%)	(199/203) 98.0% (95.0 – 99.5%)	(182/185) 98.4% (95.3 – 99.7%)	(216/226) 95.6% (92.0 – 97.9%)

a. Point estimates are as indicated. Confidence intervals are Fisher-Exact 95% CI.
b. n = 537. Nine specimens QNS for Xpert testing; 17 specimens indeterminate upon initial and retest; consensus on CIN2 vs. CIN3 status not reached for one specimen.
c. n = 555. Eight specimens indeterminate upon initial and retest; consensus on CIN2 vs. CIN3 status not reached for one specimen.
d. n = 563. Consensus on CIN2 vs. CIN3 status not reached for one specimen.
e. n = 561. Two specimens indeterminate upon initial and retest; consensus on CIN2 vs. CIN3 status not reached for one specimen.

An assessment of analytical agreement in the Stage II data set demonstrated overall agreement between the Xpert HPV Assay and itself (Specimen A vs. Specimen B; n = 533 paired comparisons) of 94.6% (95% CI 92.3 – 96.3; Kappa statistic 0.88). Overall agreement between the Xpert HPV Assay (Specimen B) and comparator method 1 (n = 556 paired comparisons) was 92.4% (95% CI 89.9 – 94.5; Kappa statistic 0.83). Overall agreement between the Xpert HPV Assay (Specimen B) and comparator method 2 (n = 554 paired comparisons) was 87.4% (95% CI 84.3 – 90.0; Kappa statistic 0.73).

Clinical performance of the Xpert HPV Assay for Pap test specimen A and B, sorted by subject age group, was determined for both disease status ≥ CIN2 and ≥ CIN3. The clinical performance relative to ≥ CIN2 disease is presented in Table 4 and the clinical performance relative to ≥ CIN3 disease is presented in Table 5.

Table 4. Xpert HPV Assay Performance vs. ≥ CIN2 Disease, by Age Group

	Pap A		Pap B	
Age Group	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
20–29	95.7% (85.5 – 99.5)	25.8% (19.1 – 33.4)	95.7% (85.5 – 99.5)	32.1% (24.9 – 39.9)
30–39	91.7% (77.5 – 98.2)	46.4% (38.3 – 54.6)	94.6% (81.8 – 99.3)	44.3% (36.4 – 52.4)
40–49	88.9% (65.3 – 98.6)	44.8% (32.6 – 57.4)	88.9% (65.3 – 98.6)	45.8% (34.0 – 58.0)
50–59	71.4% (29.0 – 96.3)	62.8% (46.7 – 77.0)	71.4% (29.0 – 96.3)	64.4% (48.8 – 78.1)
≥ 60	100% (2.5 – 100)	33.3% (9.9 – 65.1)	100% (2.5 – 100)	30.8% (9.1 – 61.4)

Table 5. Xpert HPV Assay Performance vs. ≥ CIN3 Disease, by Age Group

	Pap A		Pap B	
Age Group	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
20–29	96.7% (82.8 – 99.9)	23.8% (17.7 – 30.9)	100% (88.4 – 100)	30.1% (23.4 – 37.5)
30–39	90.9% (70.8 – 98.9)	43.1% (35.5 – 51.0)	91.3% (72.0 – 98.9)	40.7% (33.3 – 48.4)
40–49	92.9% (66.1 – 99.8)	43.7% (31.9 – 56.0)	92.9% (66.1 – 99.8)	44.7% (33.3 – 56.6)
50–59	100% (39.8 – 100)	62.2% (46.5 – 76.2)	100% (39.8 – 100)	63.8% (48.5 – 77.3)
≥ 60	100% (2.5 – 100)	33.3% (9.9 – 65.1)	100% (2.5 – 100)	30.8% (9.1 – 61.4)

A second clinical study was conducted to assess the performance of the Xpert HPV Assay in populations that more closely resemble the intended use populations served by organized cervical cancer screening programs. This study was a multicenter, method comparison study relying on residual specimens collected in PreservCyt obtained from women 20–60 years of age participating in organized cervical cancer screening programs in the UK. With rare exception, all of the specimens collected in this study were collected with a broom-like device per the ThinPrep Package Insert. The same two comparator methods were included in this study, with comparator method 1 as the primary comparator method and comparator method 2 as the secondary comparator method. Sample sizes for the study were calculated for two age groups (women ages 20–29 and women ages 30–60) that would support agreement assessment (with 95% CI) and calculation of a Kappa statistic (with 95% CI) relative to each comparator method.

In this study, residual specimens with cytology evaluation results were split into three aliquots for assessment with the Xpert HPV Assay and comparator methods 1 and 2. The sequence of aliquot removal for analysis with the Xpert HPV and comparator method 1 was randomized such that ~50% of the first aliquots were used for Xpert HPV analysis and 50% of the first aliquots were used for comparator method 1. The third aliquot was always reserved for analysis with comparator method 2. Regardless of aliquot sequence, the source specimen vial was mixed before the removal of each aliquot to ensure specimen homogeneity. Analysis with the comparator methods was completed per the respective CE-IVD Package Inserts, which procedurally, were identical to the US-IVD Package Inserts; analysis of results utilizes the cutoff parameters from the US-IVD Package Inserts.

An analysis of study data demonstrated substantial agreement between the Xpert HPV Assay and comparator method 1. This agreement is independent of subject age category (ages 20–29 and ages 30–60) and cytology status [normal (NILM, Negative for Intraepithelial Lesion or Malignancy) and worse than normal (worse than NILM)]. A summary of agreement between the Xpert HPV Assay and comparator method 1 is shown in Table 6.

Table 6. Agreement between Xpert HPV Assay and Comparator Method 1

Agreement Comparison	n	Positive Percent Agreement	Negative Percent Agreement	Overall Percent Agreement	Kappa Statistic
Overall[a]	3,438	90.4% (87.9 – 92.6%)	97.1% (96.4 – 97.7%)	95.8% (95.1 – 96.5%)	0.87 (0.85 – 0.89)
Ages 20–29	829	92.9% (89.7 – 95.4%)	94.9% (92.5 – 96.7%)	94.1% (92.3 – 95.6%)	0.88 (0.84 – 0.91)
Ages 30–60	2,609	87.8% (83.8 – 91.2%)	97.6% (96.9 – 98.2%)	96.4% (95.6 – 97.0%)	0.84 (0.81 – 0.87)
Cytology Normal	2,798	85.3% (81.0 – 88.9%)	97.4% (96.6 – 98.0%)	95.9% (95.1 – 96.6%)	0.81 (0.78 – 0.84)
Cytology > Normal	441	96.7% (93.9 – 98.4%)	90.8% (84.9 – 95.0%)	94.8% (92.3 – 96.7%)	0.88 (0.83 – 0.93)

a. Point estimates are as indicated. Confidence intervals are Fisher-Exact 95% CI.

An analysis of study data demonstrates good agreement between the Xpert HPV Assay and comparator method 2. This agreement is independent of subject age category (ages 20–29 and ages 30–60) and cytology status [normal (NILM) and worse than normal (worse than NILM)]. A summary of agreement between Xpert HPV Assay and comparator method 2 is shown in Table 7.

Table 7. Agreement between Xpert HPV Assay and Comparator Method 2

Agreement Comparison	n	Positive Percent Agreement	Negative Percent Agreement	Overall Percent Agreement	Kappa statistic
Overall[a]	3,313	84.5% (81.5 – 87.1%)	96.3% (95.5 – 97.0%)	93.9% (93.0 – 94.7%)	0.81 (0.79 – 0.84)
Ages 20–29	835	94.2% (91.1 – 96.5%)	93.1% (90.5 – 95.1%)	93.5% (91.6 – 95.1%)	0.87 (0.83 – 0.90)
Ages 30–60	2,478	75.5% (70.7 – 79.9%)	97.1% (96.3 – 97.8%)	94.0% (93.0 – 94.9%)	0.75 (0.71 – 0.79)
Cytology normal	2,798	76.9% (72.3 – 82.2%)	96.5% (95.5 – 97.2%)	94.0% (93.0 – 95.0%)	0.73 (0.69 – 0.77)
Cytology > normal	441	92.5% (89.0 – 95.1%)	93.5% (87.6 – 97.2%)	92.7% (89.9 – 95.0%)	0.83 (0.77 – 0.88)

a. Point estimates are as indicated. Confidence intervals are Fisher-Exact 95% CI.

As an additional measure of analytical agreement, the HPV positivity rate by cytology status was assessed in this study. In similar-sized samples of specimens assessed by each method, the HPV positivity rates reported by the three HPV methods are similar and in general agreement with HPV positivity rates reported in other low disease prevalence populations (e.g., the ALTS Study). A summary of the HPV positivity rates as measured by each method according to cytology status is shown in Table 8.

Table 8. HPV Positivity by Method and Cytology Status

	Xpert HPV Assay			Comparator Method 1			Comparator Method 2		
Category (UK/US)	**Total**	**Pos**	**% Pos**	**Total**	**Pos**	**% Pos**	**Total**	**Pos**	**% Pos**
Normal / NILM	3,003	383	12.8	2,968	363	12.2	2,882	366	12.7
Borderline / ASC-US	219	113	51.6	218	110	50.5	221	123	55.7
Low-grade dyskaryosis (mild) / LSIL[a]	151	118	78.1	151	121	80.1	152	129	84.9
High-grade dyskaryosis (moderate) / HSIL[b]	30	30	100.0	29	28	96.6	31	31	100.0
High-grade dyskaryosis (severe) / HSIL	36	36	100.0	36	35	97.2	36	36	100.0
Other	17	11	64.7	17	11	64.7	17	10	58.8
Total	3,456	691	20.0	3,419	668	19.5	3,339	695	20.8

a. Low grade squamous intraepithelial lesion.
b. High grade squamous intraepithelial lesion.

A subset [249/3538 (7.8%)] of the specimens enrolled in this study was pretreated with Glacial Acetic Acid (GAA) prior to HPV assessment with the Xpert HPV Assay and the comparator methods. One site utilized a modified version of a commercial methodology [71/1169 (6.1%)]; CytoLyt, Hologic, Crawley, UK, EU), while the other two sites used laboratory developed procedures based on the Espostis method [153/1170 (13.1%) and 25/1198 (2.1%), respectively].[11–13] The Xpert HPV Assay demonstrates good agreement to the comparator methods independent of GAA pretreatment status. See Table 9 and Table 10.

Table 9. Agreement between Xpert HPV Assay and Comparator Method 1 in GAA Pretreated Specimens[a]

Agreement Comparison	n	Positive Percent Agreement	Negative Percent Agreement	Overall Percent Agreement	Kappa Statistic
GAA Pretreated	243	94.2% (85.8 – 98.4%)	96.6% (92.6 – 98.7%)	95.9% (92.6 – 98.0%)	0.90 (0.84 – 0.96)
Untreated	3,180	89.7% (87.0 – 92.0%)	97.2% (96.5 – 97.8%)	95.8% (95.0 – 96.5%)	0.86 (0.84 – 0.89)

a. Point estimates are as indicated. Confidence intervals are Fisher-Exact 95% CI.

Table 10. Agreement between Xpert HPV Assay and Comparator Method 2 in GAA Pretreated Specimens[a]

Agreement Comparison	n	Positive Percent Agreement	Negative Percent Agreement	Overall Percent Agreement	Kappa Statistic
GAA Pretreated	246	87.7% (97.9 – 94.2%)	94.2% (89.6 – 97.2%)	92.3% (88.2 – 95.3%)	0.82 (0.74 – 0.90)
Untreated	3,067	84.1% (81.0 –86.9%)	96.5% (95.7 – 97.2%)	94.0% (93.1 – 94.8%)	0.81 (0.78 – 0.84)

a. Point estimates are as indicated. Confidence intervals are Fisher-Exact 95% CI.

17 Limit of Detection

The analytical sensitivity or limit of detection (LoD) of the Xpert HPV Assay was assessed using:

1. HPV positive cell lines: HPV 16 (SiHa), HPV 18 (HeLa S3), HPV 45 (MS751) and HPV 68 (ME180) in PreservCyt solution containing an HPV negative cell line (C33A) background, and
2. DNA plasmids of the 14 targeted high risk HPV types in a background of human female genomic DNA.

17.1 HPV Positive Cell Lines

The limit of detection (LoD) for HPV 16, HPV 18, HPV 45, and HPV 68 was estimated by running replicates of 20 at a minimum of six concentrations for each of the cell lines using one reagent lot of the Xpert HPV Assay. LoDs were estimated by probit analysis. The claimed LoDs were confirmed by analyzing at least 20 replicates diluted to the estimated LoD concentrations using three reagent lots of the Xpert HPV Assay. The claimed LoD is defined as the concentration at which 95% of at least 20 replicates per reagent lot are positive (Table 11).

17.2 HPV DNA Plasmids

The limit of detection (LoD) for 14 high risk HPV DNA plasmids was confirmed with a minimum of 60 replicates across two operators and three reagent lots. Tests were run on different days. The level (in copies per PCR reaction) at which the overall true positive rate is statistically greater than 95% pooled across three reagent lots was determined for each of the HPV DNA plasmids (Table 12).

Table 11. Limit of Detection: HPV Positive Cell Lines

HPV Type	LoD Est. by Probit (Cells/mL)	95% CI	99.9% CI	Conf. Level (Cells/mL)	Reagent Lot	Pos of 20 Rep.	Ct Avg. (Target)	Ct Stdev (Target)	Overall Ct Avg. (Target)	Overall Ct Stdev (Target)	% Pos	Overall % Pos
16	71	55 – 87	52 – 127	122	Lot 1	19	35.6	1.0	35.3	1.2	95	95.0
					Lot 2	19	35.0	1.4			95	
					Lot 3	19	35.4	1.2			95	
18	46	35 – 56	33 – 90	53	Lot 1	20	36.0	1.2	35.6	1.1	100	96.7
					Lot 2	19	35.3	0.9			95	
					Lot 3	19	35.6	1.1			95	
45	180	150 – 211	142 – 266	173	Lot 1	19	37.0	1.2	37.1	1.1	95	96.7
					Lot 2	20	37.0	1.2			100	
					Lot 3	19	37.4	0.9			95	
68	267	231 – 304	221 – 366	366	Lot 1	20	35.9	0.6	36.0	0.6	100	96.7
					Lot 2	19	35.9	0.7			95	
					Lot 3	20	36.2	0.5			100	

Table 12. Limit of Detection: HPV DNA Plasmids

Target	Copy Level Tested	Sample Count	FN	% Pos	Lower 1-sided 95% CI	Ct Grand Avg.	Ct Stdev
HPV 35	15	60	0	100	95.1%	33.9	0.426
HPV 39	20	60	0	100	95.1%	36.5	0.352
HPV 45	10	100	0	100	97.0%	35.6	0.533
HPV 51	10	100	0	100	97.0%	35.1	0.587
HPV 52	15	60	0	100	95.1%	34.7	0.543
HPV 56	15	101	0	100	97.1%	36.6	0.525
HPV 58	20	60	0	100	95.1%	33.7	0.412
HPV 59	10	100	0	100	97.0%	35.1	0.618
HPV 66	30	60	0	100	95.1%	36.6	0.33
HPV 68	15	100	0	100	97.0%	36.9	0.445
HPV 16	10	100	0	100	97.0%	35.1	0.559
HPV 18	10	141	1	99.3	96.7%	35.9	0.585
HPV 31	10	100	0	100	97.0%	34.2	0.529
HPV 33	10	100	0	100	97.0%	35.0	0.642

18 Assay Precision and Reproducibility

Precision and reproducibility of the Xpert HPV Assay was assessed in a 12-day, multicenter study in which two operators at each of three sites blindly tested two times per day a 16-member precision panel. This panel was composed of both contrived samples (cultured cells containing different types of HPV in a background of non-HPV-containing cultured cells) and pooled clinical specimens in PreservCyt. Each site utilized a different configuration of GeneXpert System (one site used only GX IVs, one site used a GX XVI, and one site used an Infinity 80). Three lots of Cepheid HPV Assay were used for each four-day period of study testing. At the end of the study, each member of the precision panel was assessed 144 times. Data are summarized by assay channel, represented as 16 for the HPV 16 channel, 18/45 for the HPV 18 and HPV 45 channel, 31 for the HPV 31 and other types channel, 51 for the HPV 51 and HPV 59 channel, and 39 for the HPV 39 and other types channel. See Table 13 and Table 14.

Table 13. Xpert HPV Assay Precision and Reproducibility: Panel Description and Positive Agreement [a, b]

Specimen (Target and Relative Concentration)	Assay Channel	Site 1		Site 2		Site 3		Total Agreement
		Op1	Op2	Op1	Op2	Op1	Op2	
Contrived Specimen (HPV 16 High Negative)	16	83.3% (20/24)	91.7% (22/24)	87.5% (21/24)	82.6% (19/23)	100% (23/23)	83.3% (20/24)	88.0% (125/142)
	18/45	100% (24/24)	100% (24/24)	100% (24/24)	100% (23/23)	100% (23/23)	100% (24/24)	100% (142/142)
	31	100% (24/24)	100% (24/24)	100% (24/24)	100% (23/23)	100% (23/23)	100% (24/24)	100% (142/142)
	51	100% (24/24)	100% (24/24)	100% (24/24)	100% (23/23)	100% (23/23)	100% (24/24)	100% (142/142)
	39	100% (24/24)	100% (24/24)	100% (24/24)	100% (23/23)	100% (23/23)	100% (24/24)	100% (142/142)

Table 13. Xpert HPV Assay Precision and Reproducibility: Panel Description and Positive Agreement (Continued)[a, b]

Specimen (Target and Relative Concentration)	Assay Channel	Site 1		Site 2		Site 3		Total Agreement
		Op1	Op2	Op1	Op2	Op1	Op2	
Contrived Specimen (HPV 16 Low Positive)	16	87.5% (21/24)	95.7% (22/23)	95.8% (23/24)	100% (23/23)	95.8% (23/24)	95.8% (23/24)	95.1% (135/142)
	18/45	100% (24/24)	100% (23/23)	100% (24/24)	100% (23/23)	100% (24/24)	100% (24/24)	100% (142/142)
	31	100% (24/24)	100% (23/23)	100% (24/24)	100% (23/23)	100% (24/24)	100% (24/24)	100% (142/142)
	51	100% (24/24)	100% (23/23)	100% (24/24)	100% (23/23)	100% (24/24)	100% (24/24)	100% (142/142)
	39	100% (24/24)	100% (23/23)	100% (24/24)	100% (23/23)	100% (24/24)	100% (24/24)	100% (142/142)
Contrived Specimen (HPV 16 Moderate Positive)	16	100% (24/24)	100% (24/24)	100% (24/24)	100% (21/21)	95.8% (23/24)	100% (24/24)	99.3% (140/141)
	18/45	100% (24/24)	100% (24/24)	100% (24/24)	100% (21/21)	100% (24/24)	100% (24/24)	100% (141/141)
	31	100% (24/24)	100% (24/24)	100% (24/24)	100% (21/21)	100% (24/24)	100% (24/24)	100% (141/141)
	51	100% (24/24)	100% (24/24)	100% (24/24)	100% (21/21)	100% (24/24)	100% (24/24)	100% (141/141)
	39	100% (24/24)	100% (24/24)	100% (24/24)	100% (21/21)	100% (24/24)	100% (24/24)	100% (141/141)
Contrived Specimen (HPV 18 High Negtive)	16	100% (24/24)	100% (22/22)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (142/142)
	18/45	83.3% (20/24)	86.4% (19/22)	79.2% (19/24)	87.5% (21/24)	95.8% (23/24)	91.7% (22/24)	87.3% (124/142)
	31	100% (24/24)	100% (22/22)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (142/142)
	51	100% (24/24)	100% (22/22)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (142/142)
	39	100% (24/24)	100% (22/22)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (142/142)
Contrived Specimen (HPV 18 Low Positive)	16	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (144/144)
	18/45	100% (24/24)	100% (24/24)	91.7% (22/24)	95.8% (23/24)	91.7% (22/24)	100% (24/24)	96.5% (139/144)
	31	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (144/144)
	51	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (144/144)
	39	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (144/144)

Table 13. Xpert HPV Assay Precision and Reproducibility: Panel Description and Positive Agreement (Continued)[a, b]

Specimen (Target and Relative Concentration)	Assay Channel	Site 1		Site 2		Site 3		Total Agreement
		Op1	Op2	Op1	Op2	Op1	Op2	
Contrived Specimen (HPV 18 Moderate Positive)	16	100% (24/24)	100% (23/23)	100% (23/23)	100% (24/24)	100% (24/24)	100% (23/23)	100% (141/141)
	18/45	100% (24/24)	100% (23/23)	100% (23/23)	100% (24/24)	100% (24/24)	100% (23/23)	100% (141/141)
	31	100% (24/24)	100% (23/23)	100% (23/23)	100% (24/24)	100% (24/24)	100% (23/23)	100% (141/141)
	51	100% (24/24)	100% (23/23)	100% (23/23)	100% (24/24)	100% (24/24)	100% (23/23)	100% (141/141)
	39	100% (24/24)	100% (23/23)	100% (23/23)	100% (24/24)	100% (24/24)	100% (23/23)	100% (141/141)
Contrived Specimen (HPV 68 High Negative)	16	100% (22/22)	100% (22/22)	100% (24/24)	100% (23/23)	100% (24/24)	100% (24/24)	100% (139/139)
	18/45	100% (22/22)	100% (22/22)	100% (24/24)	100% (23/23)	100% (24/24)	100% (24/24)	100% (139/139)
	31	100% (22/22)	100% (22/22)	100% (24/24)	100% (23/23)	100% (24/24)	100% (24/24)	100% (139/139)
	51	100% (22/22)	100% (22/22)	100% (24/24)	100% (23/23)	100% (24/24)	100% (24/24)	100% (139/139)
	39	90.9% (20/22)	95.5% (21/22)	100% (24/24)	91.3% (21/23)	91.7 (22/24)	91.7 (22/24)	93.5% (130/139)
Contrived Specimen (HPV 68 Low Positive)	16	100% (24/24)	100% (24/24)	100% (23/23)	100% (23/23)	100% (23/23)	100% (24/24)	100% (141/141)
	18/45	100% (24/24)	100% (24/24)	100% (23/23)	100% (23/23)	100% (23/23)	100% (24/24)	100% (141/141)
	31	100% (24/24)	100% (24/24)	100% (23/23)	100% (23/23)	100% (23/23)	100% (24/24)	100% (141/141)
	51	100% (24/24)	100% (24/24)	100% (23/23)	100% (23/23)	100% (23/23)	100% (24/24)	100% (141/141)
	39	95.8% (23/24)	95.8% (23/24)	100% (23/23)	87.0% (20/23)	100% (23/23)	100% (24/24)	96.5% (136/141)
Contrived Specimen (HPV 68 Moderate Positive)	16	100% (22/22)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (142/142)
	18/45	100% (22/22)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (142/142)
	31	100% (22/22)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (142/142)
	51	100% (22/22)	100% (24/24)	100% (24/24)	95.8% (23/24)	100% (24/24)	100% (24/24)	100% (142/142)
	39	100% (22/22)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	95.8% (23/24)	99.3% (141/142)

Table 13. Xpert HPV Assay Precision and Reproducibility: Panel Description and Positive Agreement (Continued)[a, b]

Specimen (Target and Relative Concentration)	Assay Channel	Site 1		Site 2		Site 3		Total Agreement
		Op1	Op2	Op1	Op2	Op1	Op2	
Contrived Specimen (HPV 16/45/68 Low Positive)	16	100% (24/24)	100% (23/23)	95.8% (23/24)	95.8% (23/24)	95.7% (22/23)	100% (24/24)	97.9% (139/142)
	18/45	87.5% (21/24)	95.7% (22/23)	79.2% (19/24)	87.5% (21/24)	95.7% (22/23)	95.8% (23/24)	90.1% (128/142)
	31	100% (24/24)	100% (23/23)	100% (24/24)	100% (24/24)	100% (23/23)	100% (24/24)	100% (142/142)
	51	100% (24/24)	100% (23/23)	100% (24/24)	95.8% (23/24)	100% (23/23)	100% (24/24)	99.3% (141/142)
	39	91.7% (22/24)	95.7% (22/23)	91.7% (22/24)	91.7% (22/24)	95.7% (22/23)	95.8% (23/24)	93.7% (133/142)
Contrived Specimen (Negative)	16	100% (24/24)	100% (24/24)	100% (22/22)	100% (24/24)	100% (23/23)	100% (23/23)	100% (140/140)
	18/45	100% (24/24)	100% (24/24)	100% (22/22)	100% (24/24)	100% (23/23)	100% (23/23)	100% (140/140)
	31	100% (24/24)	100% (24/24)	100% (22/22)	100% (24/24)	100% (23/23)	100% (23/23)	100% (140/140)
	51	100% (24/24)	100% (24/24)	100% (22/22)	100% (24/24)	100% (23/23)	100% (23/23)	100% (140/140)
	39	100% (24/24)	100% (24/24)	100% (22/22)	100% (24/24)	100% (23/23)	100% (23/23)	100% (140/140)
Pooled Clinical Specimen (HPV 16, HPV 31)	16	50.0% (12/24)	20.8% (5/24)	33.3% (8/24)	18.2% (4/22)	8.3% (2/24)	20.8% (5/24)	25.4% (36/142)
	18/45	100% (24/24)	100% (24/24)	100% (24/24)	100% (22/22)	100% (24/24)	100% (24/24)	100% (142/142)
	31	20.8% (5/24)	41.7% (10/24)	37.5% (9/24)	50.0% (11/22)	20.8% (5/24)	33.3% (8/24)	33.8% (48/142)
	51	100% (24/24)	100% (24/24)	100% (24/24)	100% (22/22)	100% (24/24)	100% (24/24)	100% (142/142)
	39	100% (24/24)	100% (24/24)	100% (24/24)	100% (22/22)	100% (24/24)	100% (24/24)	100% (142/142)
Pooled Clinical Specimen (HPV 18, HPV 39)	16	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (144/144)
	18/45	16.7% (4/24)	20.8% (5/24)	41.7% (10/24)	25.0% (6/24)	12.5% (3/24)	20.8% (5/24)	22.9% (33/144)
	31	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (144/144)
	51	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (144/144)
	39	4.2% (1/24)	4.2% (1/24)	0% (0/24)	8.3% (2/24)	0% (0/24)	0% (0/24)	2.8% (4/144)

Table 13. Xpert HPV Assay Precision and Reproducibility: Panel Description and Positive Agreement (Continued)[a, b]

Specimen (Target and Relative Concentration)	Assay Channel	Site 1		Site 2		Site 3		Total Agreement
		Op1	Op2	Op1	Op2	Op1	Op2	
Pooled Clinical Specimen (HPV 42, HPV 51, HPV 59)	16	100% (24/24)	100% (24/24)	100% (24/24)	100% (23/23)	95.8% (23/24)	100% (24/24)	99.3% (142/143)
	18/45	100% (24/24)	100% (24/24)	100% (24/24)	100% (23/23)	100% (24/24)	100% (24/24)	100% (143/143)
	31	100% (24/24)	100% (24/24)	100% (24/24)	100% (23/23)	100% (24/24)	100% (24/24)	100% (143/143)
	51	25.0% (6/24)	33.3% (8/24)	29.2% (7/24)	34.8% (8/23)	12.5% (3/24)	16.7% (4/24)	25.2% (36/143)
	39	100% (24/24)	100% (24/24)	100% (24/24)	100% (23/23)	100% (24/24)	100% (24/24)	100% (143/143)
Pooled Clinical Specimen (HPV 52)	16	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (23/23)	100% (23/23)	100% (142/142)
	18/45	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (23/23)	100% (23/23)	100% (142/142)
	31	20.8% (5/24)	41.7% (10/24)	33.3% (8/24)	41.7% (10/24)	8.7% (2/23)	30.4% (7/23)	29.6% (42/142)
	51	95.8% (23/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (23/23)	100% (23/23)	100% (142/142)
	39	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (23/23)	100% (23/23)	100% (142/142)
Pooled Clinical Specimen (Negative)	16	100% (24/24)	100% (24/24)	100% (24/24)	100% (22/22)	100% (24/24)	100% (24/24)	100% (142/142)
	18/45	100% (24/24)	100% (24/24)	100% (24/24)	100% (22/22)	100% (24/24)	100% (24/24)	100% (142/142)
	31	100% (24/24)	100% (24/24)	100% (24/24)	100% (22/22)	100% (24/24)	100% (24/24)	100% (142/142)
	51	100% (24/24)	100% (24/24)	100% (24/24)	100% (22/22)	100% (24/24)	100% (24/24)	100% (142/142)
	39	100% (24/24)	100% (24/24)	100% (24/24)	100% (22/22)	100% (24/24)	100% (24/24)	100% (142/142)

a. Agreement for negative and high negative specimens is shown as % negative; low and moderate positive specimen agreement shown as % positive.

b. Study included 34 total indeterminates: HPV 16 high neg(2); HPV 16 low pos(2); HPV 18 mod pos(3); HPV 18 high neg(3); HPV 18 mod pos(3); HPV 68 high neg(5); HPV 68 low pos(3); HPV 68 mod pos(2); HPV 16, 45, 68(2); CP-negative(4); HPV 16, 31(2); HPV 42, 51, 59 (1); HPV 52(2); PC-negative(2).

Table 14. Xpert HPV Assay Reproducibility: Ct Variability for Panel Members[a]

Specimen (Target and Relative Concentration)	Assay Channel (Specific Analyte)	n[b]	Mean Ct	Between Sites		Between Operators		Between Lots		Between Day		Within Assay		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Contrived Specimen (HPV 16 High Negative)	16 (16)	12	38.4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Contrived Specimen (HPV 16 Low Positive)	16 (16)	135	35.4	0	0	0.605	1.7	0.425	1.2	0	0	1.003	2.8	1.246	3.5
Contrived Specimen (HPV 16 Moderate Positive)	16 (16)	140	34.0	0	0	0.288	0.8	0.211	0.6	0	0	0.972	2.9	1.036	3.0
Contrived Specimen (HPV 18 High Negative)	18/45 (18)	22	39.2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Contrived Specimen (HPV 18 Low Positive)	18/45 (18)	139	35.9	0	0	0.408	1.1	0.414	1.2	0	0	1.149	3.2	1.287	3.6
Contrived Specimen (HPV 18 Moderate Positive)	18/45 (18)	140	34.1	0	0	0	0	0.430	1.3	0.170	0.5	1.049	3.1	1.146	3.4
Contrived Specimen (HPV 68 High Negative)	39 (68)	116	39.5	0	0	0.811	2.1	0.296	0.7	0	0	1.025	2.6	1.340	3.4
Contrived Specimen (HPV 68 Low Positive)	39 (68)	141	36.2	0.055	0.2	0.362	1.0	0.099	0.3	0.265	0.7	0.703	1.9	0.843	2.3
Contrived Specimen (HPV 68 Moderate Positive)	39 (68)	142	34.7	0	0	0.060	0.2	0.196	0.6	0	0	0.789	2.3	0.815	2.3
Contrived Specimen (HPV 16/45/68 Low Positive)	16 (16)	140	35.4	0.042	0.1	0.497	1.4	0.124	0.4	0	0	1.171	3.3	1.278	3.6
	18/45 (45)	133	37.2	0	0	0	0	0.454	1.2	0	0	1.586	4.3	1.649	4.4
	39 (68)	141	36.4	0.056	0.2	0	0	0	0	0.280	0.8	0.876	2.4	0.922	2.5
Contrived Specimen (Negative)	Negative (HMBS)	140	28.9	0.126	0.4	0.323	1.1	0.115	0.4	0	0	0.714	2.5	0.802	2.8
Pooled Clinical Specimen (HPV 16, HPV 31)	16 (16)	41	37.5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	31 (31)	97	38.2	0	0	0	0	0.356	0.9	0.453	1.2	1.411	3.7	1.524	4.0
Pooled Clinical Specimen (HPV 18, HPV 39)	18 (16)	47	39.7	0.643	1.6	0	0	0	0	1.148	2.9	1.388	3.5	1.913	4.8
	39 (39)	61	39.8	0	0	0.741	1.9	0	0	0	0	1.197	3.0	1.408	3.5
Pooled Clinical Specimen (HPV 42, HPV 51, HPV 59)	ND (42)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	51 (51)	92	38.9	0.452	1.2	0	0	0	0	0.088	0.2	1.348	3.5	1.424	3.7
	59 (59)	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pooled Clinical Specimen (HPV 52)	31 (52)	82	38.2	0.307	0.8	0	0	0	0	0	0	2.738	7.2	2.756	7.2
Pooled Clinical Specimen (Negative)	Negative (HMBS)	142	33.3	0.132	0.4	0	0	0.559	1.7	0	0	0.876	2.6	1.047	3.1

a. NA indicates insufficient continuous data to perform an ANOVA analysis.
b. Results with non-zero Ct values out of 144.

19 Analytical Specificity

A panel of 47 organisms, including bacteria, fungi, and viruses commonly found in the female urogenital tract, as well as 12 closely related Human Papilloma virus types, were tested with the Xpert HPV Assay. All organisms were spiked into HPV negative cells (C33A) in PreservCyt solution and into HPV negative cells spiked with HPV 16 positive cells (SiHa) at three times the limit of detection. The organisms and test concentrations are listed in Table 15. The analytical specificity was 100% and none of the organisms interfered with detection of HPV 16.

Table 15. Analytical Specificity Panel

Organism	Test Concentration	Organism	Test Concentration
Bacteriodes fragilis	1 x 10^8 CFU/mL	*Streptococcus agalactiae*	1 x 10^8 CFU/mL
Bifidobacterium adolescentis	1 x 10^8 CFU/mL	*Streptococcus pyogenes*	3 x 10^6 CFU/mL
Bifidobacterium breve	1 x 10^8 CFU/mL	*Trichomonas vaginalis*	1 x 10^6 CFU/mL
Candida albicans	4 x 10^6 cells/mL	Adenovirus	1 x 10^6 $TCID_{50}$/mL
Candida glabrata	1 x 10^8 cells/mL	Cytomegalovirus (CMV)	1 x 10^7 copies/mL
Chlamydia trachomatis	1 x 10^8 EB[a]/mL	Epstein Barr virus (EBV)	1 x 10^7 copies/mL
Clostridium perfringens	3 x 10^7 CFU/mL	Hepatitis B virus (HBV)	3.6 x 10^6 IU/mL
Corynebacterium xerosis	1 x 10^7 cells/mL	Hepatitis C virus (HCV)	7.62 x 10^2 IU/mL
Enterobacter cloacae	1 x 10^8 CFU/mL	Human immunodeficiency virus 1 (HIV-1)	1 x 10^6 copies/mL
Enterococcus faecalis	1 x 10^8 CFU/mL	Herpes simplex virus 1 (HSV-1)	1 x 10^7 copies/mL
Escherichia coli	1 x 10^8 CFU/mL	Herpes simplex virus 2 (HSV-2)	1 x 10^7 copies/mL
Fusobacterium nucleatum	8.7 x 10^7 CFU/mL	Human papillomavirus (HPV) 6	1.25 x 10^7 copies/mL
Klebsiella pneumoniae	1 x 10^8 CFU/mL	HPV 11	1.25 x 10^7 copies/mL
Lactobacillus acidophilus	1 x 10^7 cells/mL	HPV 26	1.25 x 10^7 copies/mL
Lactobacillus crispatus	1 x 10^7 cells/mL	HPV 30	1.25 x 10^7 copies/mL
Lactobacillus delbrueckii	1 x 10^7 cells/mL	HPV 34	1.25 x 10^7 copies/mL
Lactobacillus jensenii	3 x 10^7 CFU/mL	HPV 53	1.25 x 10^7 copies/mL
Neisseria gonorrhoeae	1 x 10^8 CFU/mL	HPV 67	1.25 x 10^7 copies/mL
Peptostreptococcus anaerobius	1 x 10^8 CFU/mL	HPV 69	1.25 x 10^7 copies/mL
Proteus mirabilis	1 x 10^8 CFU/mL	HPV 70	1.25 x 10^7 copies/mL
Proteus vulgaris	1 x 10^8 CFU/mL	HPV 73	1.25 x 10^7 copies/mL
Pseudomonas aeruginosa	1 x 10^8 CFU/mL	HPV 82	1.25 x 10^7 copies/mL
Staphylococcus aureus	1 x 10^8 CFU/mL	HPV 85	1.25 x 10^7 copies/mL
Staphylococcus epidermidis	3 x 10^6 CFU/mL		

a. Elementary Bodies.

20 Interfering Substances

Potentially interfering endogenous and exogenous substances that may be present in cervical specimens were evaluated relative to the performance of the Xpert HPV assay. Substances were individually diluted into HPV negative cells spiked with HPV 16 positive cells (SiHa) at three times the limit of detection. The substances and test concentrations are listed in Table 16. Interference was observed with whole blood (0.25% v/v) in the test sample, but not with any of the other endogenous substances at the given test concentrations. Interference was not observed with any of the exogenous substances at the given test concentrations, except for Vagisil anti-itch cream (0.25% w/v) and Vagi Gard Moisturizing Gel (0.5% w/v). Thick creams may result in pressure aborts at concentrations above 0.25% w/v in the test sample.

Table 16. Potentially Interfering Substances

Substance	Concentration
Whole blood	0.25% v/v
Mucus	0.15% v/v
Leukocytes (PBMC)	1 x 10^5 cells/mL
Vagisil Anti-Itch Cream	0.25% w/v
Clotrimazole Vaginal Cream	0.25% w/v
Preparation H Hemorrhoidal Cream	0.25% w/v
Miconazole 3	0.25% w/v
Monistat 1	0.25% w/v
Zovirax Cold Sore Cream	0.25% w/v
Vagisil Moisturizer	10% w/v
Vagi-Gard Moisturizing Gel	0.5% w/v
KY Jelly Personal Lubricant	10% w/v
Yeast Gard Douche	10% v/v
Delfen Vaginal Contraceptive Foam	10% w/v
VH Essentials Povidone-Iodine Medicated Douche	10% v/v
Norforms Feminine Deodorant Suppositories	10% w/v

21 Carry-over Contamination

A study was conducted to demonstrate that single-use, self-contained GeneXpert cartridges prevent carry-over contamination into negative samples run following very high positive samples in the same GeneXpert module. The study consisted of a negative sample processed within the same GeneXpert module immediately following a very high HPV 16 positive sample (high enough to exceed 95% of the results obtained from specimens of diseased patients in the intended use population). This testing scheme was repeated 20 times on two GeneXpert modules for a total of 42 runs resulting in 20 positive and 22 negative samples. All 20 positive samples were correctly reported as HPV 16 positive and all 22 negative samples were correctly reported as HPV negative.

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24 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

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- Lot number
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25 Table of Symbols

Symbol	Meaning
REF	Catalog number
IVD	*In vitro* diagnostic medical device
	Do not reuse
LOT	Batch code
	Consult instructions for use
	Caution
	Manufacturer
	Contains sufficient for <n> tests
CONTROL	Control
	Expiration date
CE	CE marking – European Conformity
	Temperature limitation
	Biological risks
	Warning

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