GEN-PROBE

APTIMA

APTIMA HPV 16 18/45 Genotype Assay

For *in vitro* diagnostic use.

For U.S. Export Only.

General Information

Intended Use

The APTIMA HPV 16 18/45 Genotype Assay is an *in vitro* nucleic acid amplification test for the qualitative detection of E6/E7 viral messenger RNA (mRNA) from human papillomavirus (HPV) high-risk types 16, 18, and 45 in cervical specimens from women with APTIMA HPV Assay positive results. The APTIMA HPV 16 18/45 Genotype Assay can differentiate HPV 16 from HPV 18 and/or HPV 45, but does not differentiate HPV 18 and HPV 45. Cervical specimens in ThinPrep Pap Test vials containing PreservCyt Solution may be tested with the APTIMA HPV 16 18/45 Genotype Assay, as well as cervical specimens collected with the APTIMA Cervical Specimen Collection and Transport Kit. The assay is used with the TIGRIS DTS System.

The use of the test is indicated:

1. In women 21 years and older with atypical squamous cells of undetermined significance (ASC-US) cervical cytology results, the APTIMA HPV 16 18/45 Genotype Assay can be used to test samples from women with APTIMA HPV Assay positive results to assess the presence or absence of high-risk HPV genotypes 16, 18, and/or 45. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management. The results of this test are not intended to prevent women from proceeding to colposcopy.
2. In women 30 years and older, the APTIMA HPV 16 18/45 Genotype Assay can be used to test samples from women with APTIMA HPV Assay positive results. The assay results will be used in combination with cervical cytology to assess the presence or absence of high-risk HPV genotypes 16, 18, and/or 45. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.

Summary and Explanation of the Test

Cervical cancer is one of the most common female cancers in the world. HPV is the etiological agent responsible for more than 99% of all cervical cancers.[1,2,3] HPV is a common sexually transmitted DNA virus comprised of more than 100 genotypes.[4]

The HPV viral genome is a double-stranded circular DNA approximately 7900 base pairs in length. The genome has eight overlapping open reading frames. There are six early (E) genes, two late (L) genes, and one untranslated long control region. The L1 and L2 genes encode the major and minor capsid proteins. Early genes regulate HPV viral replication. The E6 and E7 genes of high-risk HPV genotypes are known oncogenes. Proteins expressed from E6/E7 polycistronic mRNA alter cellular p53 and retinoblastoma protein functions, leading to disruption of cell-cycle check points and cell genome instability.[5,6]

Fourteen HPV genotypes are considered pathogenic or high-risk for the progression of cervical disease.[7] Multiple studies have linked genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 to disease progression.[2,5,8] Women with a persistent infection with one of these types have an increased risk for developing severe cervical dysplasia or cervical carcinoma.[7,9]

Studies have shown that different types of high-risk HPV confer different levels of risk for developing severe dysplasia or cervical carcinoma. World-wide, HPV types 16, 18, and 45 are associated with approximately 80% of all invasive cervical cancers.[2,10] These three types are found in 75% of all squamous carcinomas, with type 16 comprising the majority (85%) of these

infections. In adenocarcinomas, HPV types 16, 18, and 45 are found in 80-94% of cases, with types 18 and 45 comprising almost half of these infections.[2,10] The presence of HPV type 18 in early stage cervical cancer has been reported to be associated with a poor prognosis.[11] HPV types 18 and 45 are under-reported in precancerous lesions, which may be caused by the presence of occult lesions of the cervical canal inaccessible to colposcopic examination.[12] In women infected with HPV types 16 and/or 18, the cumulative risk of developing cervical disease is 10-fold higher compared to the risk for disease development due to other high-risk types.[13,14,15]

Principles of the Procedure

The APTIMA HPV 16 18/45 Genotype Assay involves three main steps, which take place in a single tube: target capture; target amplification by Transcription-Mediated Amplification (TMA);[16] and detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA).[17] The assay incorporates an Internal Control (IC) to monitor nucleic acid capture, amplification, and detection, as well as operator or instrument error.

Specimens are collected in or transferred to a tube containing Specimen Transport Media (STM) that lyses the cells, releases the mRNA, and protects it from degradation during storage. When the APTIMA HPV 16 18/45 Genotype Assay is performed, the target mRNA is isolated from the specimen by use of capture oligomers that are linked to magnetic microparticles. The capture oligomers contain sequences complementary to specific regions of the HPV mRNA target molecules as well as a string of deoxyadenosine residues. During the hybridization step, the sequence-specific regions of the capture oligomers bind to specific regions of the HPV mRNA target molecule. The capture oligomer-target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles, including the captured HPV mRNA target molecules bound to them, are pulled to the side of the reaction tube using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification inhibitors.

After target capture is complete, the HPV mRNA is amplified using TMA, which is a transcription-based nucleic acid amplification method that utilizes two enzymes, MMLV reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy of the target mRNA sequence containing a promoter sequence for T7 RNA polymerase. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template.

Detection of the amplicon is achieved by HPA using single-stranded nucleic acid probes with chemiluminescent labels that are complementary to the amplicon. The labeled nucleic acid probes hybridize specifically to the amplicon. The Selection Reagent differentiates between hybridized and unhybridized probes by inactivating the label on the unhybridized probes. During the detection step, light emitted from the labeled RNA:DNA hybrids is measured as photon signals called Relative Light Units (RLU) in a luminometer. Final assay results are interpreted based on the analyte signal-to-cutoff (S/CO) ratio.

IC is added to each reaction via the Target Capture Reagent. The IC monitors the target capture, amplification, and detection steps of the assay. The Dual Kinetic Assay (DKA) is the method used to differentiate the HPV signals and the IC signal.[18] IC and HPV 16 amplicon are detected by probes with rapid light-emission kinetics (flasher). The IC signal in each reaction is discriminated from the HPV 16 signal by the magnitude of the light emission. Amplicons specific to HPV 18 and 45 are detected using probes with relatively slower kinetics of light emission (glower).

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. For additional specific warnings and precautions related to instrumentation refer to the *TIGRIS DTS System Operator's Manual*.

Laboratory Related

C. Use only supplied or specified disposable laboratory ware.

D. Use routine laboratory precautions. Do not eat, drink, or smoke in designated work areas. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

E. **Warning: Irritants and Corrosives:** Avoid contact of Auto Detect 1 and Auto Detect 2 with skin, eyes and mucous membranes. If these fluids come into contact with skin or eyes, wash the affected area with water. If these fluids spill, dilute the spill with water before wiping it dry.

F. Work surfaces, pipettes, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Refer to *Test Procedure* for more information.

Specimen Related

G. Maintain proper temperature conditions during specimen shipping and storage to ensure the integrity of the specimen. Specimen stability has not been evaluated under shipping conditions other than those recommended.

H. Expiration dates listed on specimen collection/transfer kits and tubes pertain to the transfer site and not the testing facility. Specimens collected/transferred any time prior to these expiration dates are valid for testing provided they have been transported and stored in accordance with the appropriate package insert, even if these expiration dates have passed.

I. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this procedure.

J. Avoid cross-contamination during the specimen handling steps. Ensure that specimen containers do not contact one another, and discard used materials without passing over open containers. Change gloves if they come in contact with specimen.

K. Upon piercing, liquid can discharge from tube caps under certain conditions. Refer to the *Test Procedure* for more information.

L. ThinPrep liquid cytology and Cervical Specimen Collection and Transport (CSCT) specimens should be rejected if a collection device has been left in the sample tube.

Assay Related

M. Store reagents at the specified temperatures. Performance of the assay may be affected by use of improperly stored reagents.

N. Avoid microbial and ribonuclease contamination of reagents.

O. Do not use kit after its expiration date.

P. Do not interchange, mix, or combine assay reagents or Calibrators from kits with different lot numbers.

Q. APTIMA Assay Fluids, APTIMA System Fluid Preservative, and Auto Detect Reagents are not part of the Master Lot; any lot may be used.

R. Thorough mixing of assay reagents is necessary to achieve accurate assay results.

S. Tips with hydrophobic plugs must be used.

Reagent Storage and Handling Requirements

Do not use reagents beyond the expiration date indicated on the vials. See below for additional storage instructions.

A. The following reagents are stored at 2°C to 8°C (refrigerated) upon receipt:

HPV 16 18/45 Amplification Reagent

HPV 16 18/45 Enzyme Reagent

HPV 16 18/45 Probe Reagent

HPV 16 18/45 Internal Control Reagent

HPV 16 18/45 Positive Calibrators and HPV 16 18/45 Negative Calibrators

B. The following reagents are stored at 15°C to 30°C (room temperature):

HPV 16 18/45 Amplification Reconstitution Solution

HPV 16 18/45 Enzyme Reconstitution Solution

HPV 16 18/45 Probe Reconstitution Solution

HPV 16 18/45 Target Capture Reagent

HPV 16 18/45 Selection Reagent

Wash Solution

Oil Reagent

Buffer for Deactivation Fluid

Auto Detect Reagent 1

Auto Detect Reagent 2

APTIMA System Fluid Preservative

C. After reconstitution, the following reagents are stable for 30 days when stored at 2°C to 8°C:

HPV 16 18/45 Amplification Reagent

HPV 16 18/45 Enzyme Reagent

HPV 16 18/45 Probe Reagent

D. Working Target Capture Reagent (wTCR) is stable for 30 days when stored at 15°C to 30°C. Do not refrigerate.

E. Discard any unused reconstituted reagents and wTCR after 30 days or after the Master Lot expiration date, whichever comes first.

F. The APTIMA HPV 16 18/45 Genotype Assay reagents are stable for a cumulative of 48 hours when stored on-board the TIGRIS DTS System.

G. The Probe Reagent and Reconstituted Probe Reagent are photosensitive. Store the reagents protected from light.

H. Do not freeze reagents.

Specimen Collection and Storage

A. Specimen collection and processing

ThinPrep liquid cytology specimens

1. Collect cervical specimens in ThinPrep Pap Test vials containing PreservCyt Solution with broom-type or cytobrush/spatula collection devices according to the manufacturer's instructions.
2. Prior to or after processing with the ThinPrep 2000 System or ThinPrep 3000 System, transfer 1 mL of the ThinPrep liquid cytology specimen into an APTIMA Specimen Transfer tube, according to the APTIMA Specimen Transfer Kit package insert.
3. If testing the specimen after processing using the ThinPrep 2000 System or ThinPrep 3000 System, process the ThinPrep liquid cytology specimen in accordance with the manufacturer's instructions and the APTIMA Specimen Transfer Kit package insert. Transfer 1 mL of the fluid remaining in the ThinPrep Pap Test vial into an APTIMA Specimen Transfer tube according to the instructions in the APTIMA Specimen Transfer Kit package insert.

APTIMA Cervical Specimen Collection and Transport Kit specimens

Collect the specimen according to the APTIMA CSCT Kit instructions for use.

B. Transport and storage before testing:

ThinPrep liquid cytology specimens

1. Transport the ThinPrep liquid cytology specimens at 2°C to 30°C.
2. Specimens should be transferred to an APTIMA Specimen Transfer tube within 105 days of collection.
3. Prior to transfer, ThinPrep liquid cytology specimens should be stored at 2°C to 30°C, with no more than 30 days at temperatures above 8°C.
4. ThinPrep liquid cytology specimens transferred to an APTIMA Specimen Transfer tube may be stored at 2°C to 30°C for up to 60 days.

5. If longer storage is needed, the ThinPrep liquid cytology specimen or the ThinPrep liquid cytology specimen diluted into the Specimen Transfer tube may be stored at -20°C or colder for up to 24 months.

APTIMA Cervical Specimen Collection and Transport Kit specimens

1. Transport and store specimens at 2°C to 30°C for up to 60 days.
2. If longer storage is needed, transport kit specimens may be stored at -20°C or colder for up to 24 months.

C. Specimen storage after testing:

1. Specimens that have been assayed must be stored upright in a rack.
2. Specimen tubes should be covered with a new, clean plastic or foil barrier.
3. If assayed specimens need to be frozen or shipped, remove penetrable cap and place new non-penetrable caps on the specimen tubes. If specimens need to be shipped for testing at another facility, specified temperatures must be maintained. Prior to uncapping previously tested and recapped specimens, tubes must be centrifuged for 5 minutes at 420 Relative Centrifugal Force (RCF) to bring all of the liquid down to the bottom of the tube.

Note: *Specimens must be shipped in accordance with applicable local, national and international transport regulations.*

TIGRIS DTS System

Reagents and Materials Provided

APTIMA HPV 16 18/45 Genotype Assay Kit for the TIGRIS DTS System, 100 tests
Cat No. 303234 (3 boxes)

Calibrators can be purchased separately. See individual box catalog number below.

APTIMA HPV 16 18/45 Genotype Assay Refrigerated Box
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
A	**HPV 16 18/45 Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution containing < 5% bulking agent.*	1 vial
E	**HPV 16 18/45 Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 vial
P	**HPV 16 18/45 Probe Reagent** *Non-infectious chemiluminescent DNA probes (< 500 ng/vial) dried in succinate buffered solution containing < 5% detergent.*	1 vial
IC	**HPV 16 18/45 Internal Control Reagent** *Non-infectious RNA Transcript in buffered solution containing < 5% detergent.*	1 vial

APTIMA HPV 16 18/45 Genotype Assay Room Temperature Box
(store at 15°C to 30°C upon receipt)

Symbol	Component	Quantity
AR	**HPV 16 18/45 Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 vial
ER	**HPV 16 18/45 Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 vial
PR	**HPV 16 18/45 Probe Reconstitution Solution** *Succinate buffered solution containing < 5% detergent.*	1 vial
S	**HPV 16 18/45 Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 vial
TCR	**HPV 16 18/45 Target Capture Reagent** *Non-infectious nucleic acid in a buffered solution containing solid phase (< 0.5 mg/mL).*	1 vial
	Reconstitution Collars	3
	Master Lot Barcode Sheet	1 sheet

APTIMA HPV 16 18/45 Genotype Assay Calibrators Box (Cat. No. 303235)
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
PCAL1	**HPV 16 18/45 Positive Calibrator 1** *Non-infectious HPV 18 in vitro transcript at 750 copies per mL in a buffered solution containing < 5% detergent.*	5 vials
PCAL2	**HPV 16 18/45 Positive Calibrator 2** *Non-infectious HPV 16 in vitro transcript at 1000 copies per mL in a buffered solution containing < 5% detergent.*	5 vials
NCAL	**HPV 16 18/45 Negative Calibrator** *Buffered solution containing < 5% detergent.*	5 vials

Materials Required But Available Separately

Note: *Materials available from Gen-Probe have catalog numbers listed, unless otherwise specified.*

	Cat. No.
TIGRIS DTS System	105118
APTIMA Assay Fluids Kit	302382
(APTIMA Wash Solution, APTIMA Buffer for Deactivation Fluid, and APTIMA Oil Reagent)	
APTIMA Auto Detect Kit	301048
APTIMA System Fluid Preservative Kit	302380
Tips, 1000 µl conductive, liquid sensing	10612513 (Tecan)
TIGRIS DTS System Run Kit	301191
Multi-tube Units (MTU)	*104772-02*
MTU-Tiplet Waste Bag	*900907*
MTU Waste Deflectors	*900931*
MTU Waste Covers	*105523*
APTIMA Specimen Transfer Kit	301154C
APTIMA Cervical Specimen Collection and Transport Kit	302657
APTIMA Penetrable Caps	105668
Replacement non-penetrable caps	103036
Spare Caps for Amplification and Probe Reagent reconstitution solutions	CL0041
Spare Caps for Enzyme Reagent reconstitution solution	CL0041
Spare Caps for TCR and Selection Reagent	501604
Bleach, minimum 5% or 0.7 M sodium hypochlorite solution	—
Water for the TIGRIS DTS System	—
consult the TIGRIS DTS System Operator's Manual for specifications	
Disposable gloves	—

Optional Materials

	Cat. No.
Bleach Enhancer for Cleaning	302101

Test Procedure

Note: *See the TIGRIS DTS System Operator's Manual for additional TIGRIS DTS System procedural information.*

A. Work Area Preparation

Clean work surfaces where reagents will be prepared. Wipe down work surfaces and pipettors with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Allow sodium hypochlorite solution to contact surfaces and pipettors for at least 1 minute and then follow with a water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface on which the reagents will be prepared with clean, plastic-backed absorbent laboratory bench covers.

B. Reagent Preparation of a New Kit

Note: *Reagent Reconstitution should be performed prior to beginning any work on the TIGRIS DTS System.*

1. To reconstitute Amplification, Enzyme, and Probe Reagents, combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
   a. Pair each reconstitution solution with its lyophilized reagent. Ensure that the reconstitution solution and lyophilized reagent have matching label colors before attaching the reconstitution collar.
   b. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
   c. Open the lyophilized reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 1, Step 1).
   d. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.
   e. While holding the solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 1, Step 2).
   f. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 1, Step 3).
   g. Gently swirl the solution in the vial to mix thoroughly. Avoid creating foam while swirling the vial (Figure 1, Step 4).
   h. Wait for the lyophilized reagent to go into solution, then invert the assembled bottles again, tilting at a 45° angle to minimize foaming (Figure 1, Step 5). Allow all of the liquid to drain back into the plastic bottle.
   i. Remove the reconstitution collar and vial (Figure 1, Step 6).
   j. Recap the bottle. Record operator initials and the reconstitution date on all reconstituted reagent vials (Figure 1, Step 7).
   k. Discard the reconstitution collar and vial (Figure 1, Step 8).

Warning: *Avoid creating foam when reconstituting reagents. Foam compromises the level-sensing in the TIGRIS DTS System.*

Note: *Thoroughly mix Amplification, Enzyme, Probe, and Selection Reagents by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents.*

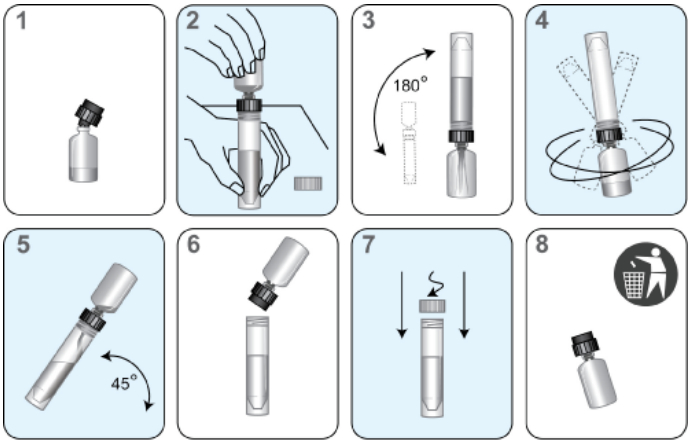

Figure 1. TIGRIS DTS System reconstitution process

2. Prepare the working Target Capture Reagent (wTCR):
   a. Pair the appropriate bottles of TCR and IC.
   b. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents are paired.
   c. Open the bottle of TCR, and set the cap on a clean, covered work surface.
   d. Open the bottle of IC and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the IC bottle.
   e. Cap the bottle of TCR and gently swirl the solution to mix the contents. Avoid creating foam during this step.
   f. Record operator initials and the current date on the label.
   g. Discard the IC bottle and cap.
   h. Precipitate may form in wTCR which may yield invalid results due to volume verification errors. Precipitate may be dissolved by warming wTCR at 42°C to 60°C for up to 90 minutes. Allow the wTCR to equilibrate to room temperature prior to use. Do not use if precipitate persists.
3. Prepare Selection Reagent
   a. Check the reagent lot number on the Master Lot Barcode Sheet to make sure it belongs to the kit.
   b. If Selection Reagent contains precipitate, warm the Selection Reagent at 60°C ± 1°C for up to 45 minutes to facilitate dissolution of precipitate. Gently mix the bottle every 5 to 10 minutes. Allow the Selection Reagent to equilibrate to room temperature prior to use. Do not use if precipitate or cloudiness persists.

Note: *Thoroughly mix by gently inverting all reagents prior to loading on the system. Avoid creating foam during inversion of reagents.*

C. Reagent Preparation for Previously Reconstituted Reagents

1. Previously reconstituted Amplification, Enzyme, and Probe Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay.
2. If reconstituted Probe Reagent contains precipitate that does not return to solution at room temperature, heat at a temperature that does not exceed 60°C for 1 to 2 minutes. Do not use if precipitate or cloudiness is present.
3. If wTCR contains precipitate, warm wTCR at 42°C to 60°C for up to 90 minutes. Allow the wTCR to equilibrate to room temperature prior to use. Do not use if precipitate persists.
4. If Selection Reagent contains precipitate, warm the Selection Reagent at 60°C ± 1°C for up to 45 minutes to facilitate dissolution of precipitate. Gently mix the bottle every 5 to 10 minutes. Allow the Selection Reagent to equilibrate to room temperature prior to use. Do not use if precipitate or cloudiness persists.
5. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents.
6. Do not top off reagent bottles. The TIGRIS DTS System will recognize and reject bottles that have been topped off.

D. Sample Handling

1. Allow the samples (calibrators and specimens) to reach room temperature prior to processing.
2. **Do not vortex samples**.
3. Inspect sample tubes before loading into the racks. If a sample tube contains bubbles or has a lower volume than is typically observed, centrifuge the tube for 5 minutes at 420 RCF to ensure that there is no liquid in the cap.

Note: *Failure to follow step 3 may result in liquid discharge from the sample tube cap.*

E. System Preparation

Set up the system and worklist according to the instructions in the *TIGRIS DTS System Operator's Manual* and the *Procedural Notes* section below.

Procedural Notes

A. Calibrators

1. Each worklist must contain 2 replicates of the Negative Calibrator and each Positive Calibrator. In order to work properly with the APTIMA HPV 16 18/45 Genotype Assay Software, the Negative Calibrator must be in the first tube position of the first rack of the worklist, Positive Calibrator 1 must be in the second tube position of the first rack of the worklist, and Positive Calibrator 2 must be in the third tube position of the first rack of the worklist.
2. Attempts to pipette more than two replicates from a calibrator tube can lead to insufficient volume errors.
3. Calibrators are to be used with the corresponding Master Lot of reagents. The operator must check to ensure that the correct lot of calibrators is used with the corresponding Master Lot of kit reagents as indicated on the Master Lot Barcode Sheet. The appropriate lot number should be referenced when ordering additional calibrators.

B. Temperature

Room temperature is defined as 15°C to 30°C.

C. Glove Powder

As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.

Quality Control Procedures

A. Run Validity Criteria

The software automatically determines run validity. The software will invalidate a run if any of the following conditions occur:

- More than one invalid Negative Calibrator replicate.
- More than one invalid Positive Calibrator 1 replicate.
- More than one invalid Positive Calibrator 2 replicate.
- More than 1 of 6 invalid calibrator replicates combined.

A run may be invalidated by an operator if technical, operator, or instrument difficulties are observed and documented while performing the assay.

An invalid run must be repeated. Aborted runs must be repeated.

B. Calibrator Acceptance Criteria

The table below defines the RLU criteria for the Negative and Positive Calibrator replicates.

Negative Calibrator	
18/45 RLU	*≥ 0 and ≤ 60,000 RLU*
IC/16 RLU	*≥ 75,000 and ≤ 300,000 RLU*
Positive Calibrator 1	
18/45 RLU	*≥ 850,000 and ≤ 2,200,000 RLU*
IC/16 RLU	*≤ 475,000 RLU*
Positive Calibrator 2	
18/45 RLU	*≤ 115,000 RLU*
IC/16 RLU	*≥ 625,000 and ≤ 4,000,000 RLU*

C. IC Cutoff

The IC cutoff is determined from the IC/16 Analyte signal from the valid Negative Calibrator replicates.

IC Cutoff = *0.5 x [mean IC/16 RLU of the valid Negative Calibrator replicates]*

D. Analyte 16 Cutoff

The analyte cutoff for HPV 16 is determined from the IC/16 RLU signal from the valid Negative Calibrator replicates and the valid Positive Calibrator 2 replicates.

$$\textbf{Analyte 16 Cutoff} = \begin{array}{l} 2 \times [\text{mean IC/16 RLU of the valid Negative Calibrator replicates}] + \\ 0.1 \times [\text{mean IC/16 RLU of the valid Positive Calibrator 2 replicates}] \end{array}$$

E. Analyte 16 Signal to Cutoff (S/CO)

The analyte S/CO for HPV 16 is determined from the IC/16 RLU signal of the test sample and the analyte 16 cutoff for the run.

$$\textbf{Analyte 16 S/CO} = \frac{\text{test sample IC/16 RLU}}{\text{analyte 16 cutoff}}$$

F. Analyte 18/45 Cutoff

The analyte cutoff for HPV 18/45 is determined from the 18/45 RLU signal from the valid Negative Calibrator replicates and the valid Positive Calibrator 1 replicates.

$$\textbf{\textit{Analyte 18/45 Cutoff}} = \textit{1 x [mean 18/45 RLU of the valid Negative Calibrator replicates] + 0.18 x [mean I8/45 RLU of the valid Positive Calibrator 1 replicates]}$$

G. Analyte 18/45 Signal to Cutoff (S/CO)

The analyte S/CO for HPV 18/45 is determined from the 18/45 RLU signal of the test sample and the analyte 18/45 cutoff for the run.

$$\textbf{\textit{Analyte 18/45 S/CO}} = \frac{\textit{test sample 18/45 RLU}}{\textit{analyte 18/45 cutoff}}$$

Test Interpretation

Test results are automatically determined by the assay software. A test result may be negative for both HPV 16 and HPV 18/45, negative for HPV 16 and positive for HPV 18/45, positive for HPV 16 and negative for HPV 18/45, positive for both HPV 16 and HPV 18/45, or invalid as determined by the IC RLU and S/CO ratios as described in the table below. A test result may also be invalid due to other parameters (e.g., abnormal curve shape) being outside the normal expected ranges. Invalid test results should be repeated.

Note: *A minimum volume of 1.7 mL is required in order to test 1 aliquot of the sample.*

APTIMA HPV 16 18/45 Genotype Assay Result	**Criteria**
Negative - 16 **Negative - 18/45**	*IC/HPV 16 RLU ≥ IC Cutoff and* *HPV 16 S/CO < 1.00 and* *HPV 18/45 S/CO < 1.00*
Negative - 16 **Positive - 18/45**	*HPV 16 S/CO < 1.00 and* *HPV 18/45 S/CO ≥ 1.00 and* *HPV 18/45 RLU ≤ 3,000,000*
Positive - 16 **Negative - 18/45**	*HPV 16 S/CO ≥ 1.00 and* *IC/HPV 16 RLU ≤ 4,000,000 and* *HPV 18/45 S/CO < 1.00*
Positive - 16 **Positive - 18/45**	*HPV 16 S/CO ≥ 1.00 and* *IC/HPV 16 RLU ≤ 4,000,000 and* *HPV 18/45 S/CO ≥ 1.00 and* *HPV 18/45 RLU ≤ 3,000,000*
Invalid	*HPV 16 S/CO < 1.00 and* *HPV 18/45 S/CO < 1.00 and* *IC/HPV 16 RLU < IC cutoff* *or* *IC/HPV 16 RLU > 4,000,000* *or* *HPV 18/45 RLU > 3,000,000*

Limitations

A. The performance of the APTIMA HPV 16 18/45 Genotype Assay has not been evaluated for HPV vaccinated individuals.

B. The APTIMA HPV 16 18/45 Genotype Assay has not been evaluated in cases of suspected sexual abuse.

C. Prevalence of HPV infection in a population may affect performance. Positive predictive values decrease when testing populations with low prevalence or individuals with no risk of infection.

D. ThinPrep liquid cytology specimens containing less than 1 mL after ThinPrep Pap Test slide preparation are considered inadequate for the APTIMA HPV 16 18/45 Genotype Assay.

E. Test results may be affected by improper specimen collection, storage, or specimen processing.

F. A negative APTIMA HPV 16 18/45 Genotype Assay result does not exclude the possibility of cytologic abnormalities or of future or underlying CIN2, CIN3, or cancer.

G. The APTIMA HPV 16 18/45 Genotype Assay provides qualitative results. Therefore, a correlation cannot be drawn between the magnitude of a positive assay signal and the expression level of mRNA in a specimen.

H. Detection of high-risk HPV (types 16, 18, and 45) mRNA 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.

I. Infection with HPV is not an indicator of cytologic HSIL or underlying high-grade CIN, nor does it imply that CIN2, CIN3, or cancer will develop. Most women infected with one or more high-risk HPV types do not develop CIN2, CIN3, or cancer.

J. The following may interfere with the performance of the assay when present at concentrations greater than those specified: vaginal lubricants (containing Polyquaternium 15) at 1% w/v, anti-fungal cream (containing tioconazole) at 0.03% w/v, mucus at 0.3% w/v, intravaginal hormones (containing progesterone) at 1% w/v, *Trichomonas vaginalis* at 3×10^4 cells/mL.

K. The effects of other potential variables such as vaginal discharge, use of tampons, etc. and specimen collection variables have not been evaluated.

L. Use of this device must be limited to personnel trained in the use of the APTIMA HPV 16 18/45 Genotype Assay.

M. Cross-contamination of samples can cause false positive results. The carry-over rate of the APTIMA HPV 16 18/45 Genotype Assay on the TIGRIS DTS System has been determined in a non-clinical study to be 0.35%.

N. The APTIMA HPV 16 18/45 Genotype Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

Expected Results: Prevalence of High-Risk HPV mRNA

The prevalence of high-risk HPV infection varies widely and is influenced by several factors, of which age is the greatest contributor.[19,20] Many studies have investigated HPV prevalence as determined by the detection of HPV DNA, however few studies report prevalence based on detection of HPV oncogenic mRNA. Women from a variety of clinical sites (n=18) representing a wide geographic distribution and a diverse population (10 states within the United States) were enrolled in a prospective clinical study known as the CLEAR trial to evaluate the APTIMA HPV Assay, which detects 14 high-risk HPV types. Samples from women in the CLEAR trial with APTIMA HPV Assay positive results were evaluated at three testing sites with the APTIMA HPV 16 18/45 Genotype Assay in a separate clinical study. The prevalence of HPV 16, 18/45, as well as the remaining 11 high-risk HPV types observed in the clinical trial, based on results of testing with the APTIMA HPV Assay and the APTIMA HPV 16 18/45 Genotype Assay, was categorized overall, by age group, and by testing site. Results are shown in Table 1 for the atypical squamous cells of undetermined significance (ASC-US) and the negative for intraepithelial lesion or malignancy (NILM) populations.

Table 1: High-risk HPV mRNA Prevalence in Populations by Age Group, Testing Site, and All Combined

	Positivity Rate % (x/n)							
	ASC-US Population (≥ 21 Years)				NILM Population (≥ 30 Years)			
	HPV 16 Pos	HPV 18/45 Pos	HPV 16 & 18/45 Pos	11 Other HR* Pos	HPV 16 Pos	HPV 18/45 Pos	HPV 16 & 18/45 Pos	11 Other HR* Pos
All	7.8 (71/912)	5.2 (47/912)	0.3 (3/912)	25.5 (233/912)	0.4 (47/10,846)	0.4 (47/10,846)	0 (0/10,846)	3.9 (421/10,846)
Age Group (years)								
21 to 29	13.2 (51/386)	4.9 (19/386)	0.5 (2/386)	38.3 (148/386)	N/A	N/A	N/A	N/A
30 to 39	5.4 (14/257)	7.0 (18/257)	0.4 (1/257)	21.8 (56/257)	0.7 (30/4,188)	0.6 (27/4,188)	0 (0/4,188)	5.3 (221/4,188)
≥ 40	2.2 (6/269)	3.7 (10/269)	0 (0/269)	10.8 (29/269)	0.3 (17/6,658)	0.3 (20/6,658)	0 (0/6,658)	3.0 (200/6,658)
Testing Site								
1	9.0 (27/301)	4.3 (13/301)	0.7 (2/301)	24.9 (75/301)	0.4 (13/3,666)	0.5 (18/3,666)	0 (0/3,666)	3.8 (141/3,666)
2	7.4 (23/310)	6.1 (19/310)	0 (0/310)	26.5 (82/310)	0.5 (18/3,671)	0.5 (17/3,671)	0 (0/3,671)	3.7 (136/3,671)
3	7.0 (21/301)	5.0 (15/301)	0.3 (1/301)	25.2 (76/301)	0.5 (16/3,509)	0.3 (12/3,509)	0 (0/3,509)	4.1 (144/3,509)

N/A = Not Applicable, HR = High-risk, Pos = Positive
* HPV types 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and 68

APTIMA HPV 16 18/45 Genotype Assay Clinical Study Design with ThinPrep Liquid Cytology Specimens

The APTIMA HPV 16 18/45 Genotype Assay was evaluated using referral cytology specimens collected from consenting women during the prospective, multicenter US clinical study known as the CLEAR trial. The CLEAR trial was conducted to determine the clinical performance of the APTIMA HPV Assay for detection of cervical intraepithelial neoplasia grade 2 or more severe cervical disease (≥CIN2). Women were enrolled into either the ASC-US Study or the NILM Study based on their referral ThinPrep liquid based cytology results from routine cervical cancer screening. The ASC-US Study population included women 21 years and older with ASC-US cytology results and the NILM Study population included women 30 years of age and older with NILM cytology results.

Women from 18 clinical sites, primarily obstetrics/gynecology clinics, which covered a wide geographic distribution and a diverse population, were analyzed. During the CLEAR trial, residual referral cytology specimens were tested with both the APTIMA HPV Assay and a commercially available HPV DNA test. For the APTIMA HPV 16 18/45 Genotype Assay clinical trial, samples from the residual referral cytology specimens were tested with the APTIMA HPV 16 18/45 Genotype Assay.

All women in the ASC-US Study were referred to colposcopy, regardless of their APTIMA HPV Assay and commercially available HPV DNA test results. An endocervical curettage (ECC) biopsy and cervical punch biopsies (1 biopsy from each of the 4 quadrants) were obtained. If a lesion was visible, a punch biopsy was obtained (directed method; 1 biopsy per lesion) and quadrants without a visible lesion were biopsied at the squamocolumnar junction (random method).

In the NILM Study, women positive with the APTIMA HPV Assay and/or the commercially available HPV DNA test, as well as randomly selected women who were negative with both assays, were referred to colposcopy for the baseline evaluation. An ECC biopsy was obtained from each woman who attended colposcopy. Punch biopsies were obtained from visible lesions only (direct method; 1 biopsy per lesion). Follow-up of women in the NILM Study who did not have ≥CIN2 at baseline is ongoing for 3 years with annual cytology visits. Women with ASC-US or more severe cytology results during the follow-up period are referred to colposcopy using the same biopsy procedure performed for the baseline evaluation.

For both the ASC-US and NILM studies, disease status was determined from a consensus histology review panel, which was based on agreement of at least 2 expert pathologists. The expert pathologists were masked to the women's HPV and cytology status, as well as each other's histology diagnoses. Investigators, clinicians, and women were masked to the APTIMA HPV Assay and commercially available HPV DNA test results until after completion of the colposcopy visit, to avoid bias.

To validate the intended use of the APTIMA HPV 16 18/45 Genotype Assay as a reflex test for an APTIMA HPV Assay positive specimen, residual referral cytology specimens from all evaluable women in the ASC-US Study and the NILM Study with an APTIMA HPV Assay positive result were eligible for testing with the APTIMA HPV 16 18/45 Genotype Assay. Clinical performance of the APTIMA HPV 16 18/45 Genotype Assay for detection of ≥CIN2 and cervical intraepithelial neoplasia grade 3 or more severe cervical disease (≥CIN3) was evaluated.

Assay Performance

ASC-US ≥ 21 Years Population: APTIMA HPV 16 18/45 Genotype Assay Clinical Performance with ThinPrep Liquid Cytology Specimens

In total, there were 400 evaluable women 21 years of age and older with ASC-US cytology results and APTIMA HPV Assay positive results whose referral cytology samples were eligible for testing with the APTIMA HPV 16 18/45 Genotype Assay. Of these, 46 women did not have sufficient referral cytology sample volume available for testing in this study and 6 had undetermined disease diagnoses; after a missing values analysis, they were not included in the performance calculations. The 348 evaluable women with conclusive disease status had valid APTIMA HPV 16 18/45 Genotype Assay results based on reflex testing from an APTIMA HPV Assay positive result. Sixty-seven (67) women had ≥CIN2 and 29 had ≥CIN3.

Of the 348 evaluable women with APTIMA HPV Assay positive results, 117 women had APTIMA HPV 16 18/45 Genotype Assay positive results indicating the presence of HPV 16 and/or HPV 18/45; 231 had negative results, indicating the presence of one or more of the other 11 high-risk HPV types as detected by the APTIMA HPV Assay (i.e., HPV types 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and 68). An additional 545 evaluable women 21 years of age and older with ASC-US cytology results had APTIMA HPV Assay negative results during the CLEAR trial. An APTIMA HPV Assay negative result indicates that none of the 14 high-risk HPV types are present, and were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis. Prevalence of ≥CIN2 and ≥CIN3 in evaluable women with ASC-US cytology results was 8.8% and 3.7% respectively. The results of the APTIMA HPV 16 18/45 Genotype Assay by APTIMA HPV Assay result and consensus histology review panel diagnosis are presented in Table 2.

Table 2: ASC-US ≥ 21 Years Population: Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay by Consensus Histology Review Panel Diagnosis

APTIMA HPV Assay Result	AHPV-GT Assay Result*	Interpretation	Consensus Histology Review Panel Diagnosis						
			Undetermined**	Normal	CIN1	CIN2	CIN3	Cancer	Total
Positive	HPV 16 Neg, HPV 18/45 Neg	Other HR HPV Pos	2	125	73	23	10	0	233
	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos	1	27	18	11	14	0	71
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos	3	23	14	3	3	1	47
	HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	0	1	0	1	1	0	3
		Total	6	176	105	38	28	1	354
Negative	HPV 16/18/45 Neg***	HR HPV Neg	13	458	75	8	4	0	558
		Total	19	634	180	46	32	1****	912

AHPV-GT = APTIMA HPV 16 18/45 Genotype Assay, CIN1 = Cervical Intraepithelial Neoplasia Grade 1, HR = High-risk, Neg = Negative, Pos = Positive

*All samples had final results (upon final testing or after resolution of initial invalids per procedure).

**19 women attended the colposcopy visit but a diagnosis could not be determined for the following reasons: < 5 biopsy specimens obtained all with histology results of normal/CIN1 (n=15), no biopsies collected (n=3), and biopsy slides lost (n=1).

***Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

****One woman had adenocarcinoma in situ (AIS).

The absolute risk of disease (≥CIN2 and ≥CIN3) by APTIMA HPV 16 18/45 Genotype Assay result and APTIMA HPV Assay result are shown in Table 3. The risk of ≥CIN2 in women with HPV types 16, 18, and/or 45 present was 29.1% compared to 14.3% in women with one or more of the other 11 high-risk HPV types present and 2.2% in women with no high-risk HPV types present. Absolute risks are shown by age group in Table 4.

Table 3: ASC-US ≥ 21 Years Population: Absolute Risk of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay

APTIMA HPV Assay Result	AHPV-GT Assay Result	Interpretation	≥CIN2	≥CIN3
			Absolute Risk (95% CI)	Absolute Risk (95% CI)
Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	29.1 (34/117) (22.4, 36.0)	16.2 (19/117) (11.4, 21.1)
	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	35.7 (25/70) (26.1, 45.9)	20.0 (14/70) (12.6, 28.0)
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	15.9 (7/44) (7.2, 28.3)	9.1 (4/44) (2.9, 19.5)
	HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	66.7 (2/3) (15.2, 98.2)	33.3 (1/3) (1.8, 84.6)
	HPV 16/18/45 Neg	Other HR HPV Pos	14.3 (33/231) (10.9, 17.9)	4.3 (10/231) (2.4, 6.8)
	Pos or Neg	HR HPV Pos	19.3 (67/348) (17.1, 21.3)	8.3 (29/348) (6.9, 9.4)
Negative	HPV 16/18/45 Neg*	HR HPV Neg	2.2 (12/545) (1.2, 3.5)	0.7 (4/545) (0.2, 1.6)
		Prevalence	8.8% (79/893)	3.7% (33/893)

AHPV-GT = APTIMA HPV 16 18/45 Genotype Assay, HR = High-risk, Pos = Positive, Neg = Negative
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

Table 4: ASC-US ≥ 21 Years Population: Absolute Risk of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay by Age Group

	APTIMA HPV Assay Result	AHPV-GT Assay Result	Interpretation	≥CIN2	≥CIN3
				Absolute Risk (95% CI)	Absolute Risk (95% CI)
21 to 29 Years	Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	26.8 (19/71) (18.3, 35.7)	15.5 (11/71) (9.3, 21.8)
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	28.0 (14/50) (17.5, 39.6)	18.0 (9/50) (9.9, 26.9)
		HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	15.8 (3/19) (3.7, 36.3)	5.3 (1/19) (0.2, 22.5)
		HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	100 (2/2) (27.0, 100)	50.0 (1/2) (2.9, 97.1)
		HPV 16/18/45 Neg	Other HR HPV Pos	17.0 (25/147) (12.6, 21.5)	5.4 (8/147) (2.8, 8.5)
		Pos or Neg	HR HPV Pos	20.2 (44/218) (17.6, 22.5)	8.7 (19/218) (7.1, 9.8)
	Negative	HPV 16/18/45 Neg*	HR HPV Neg	3.6 (6/165) (1.5, 6.9)	0.6 (1/165) (0.0, 2.7)
			Prevalence	13.1% (50/383)	5.2% (20/383)
30 to 39 Years	Positive Negative	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	32.3 (10/31) (19.0, 45.9)	16.1 (5/31) (7.0, 25.4)
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	50.0 (7/14) (24.2, 74.2)	21.4 (3/14) (5.1, 41.6)
		HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	18.8 (3/16) (3.0, 40.6)	12.5 (2/16) (1.3, 30.8)
		HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	0 (0/1) (0.0, 93.5)	0 (0/1) (0.0, 93.3)
		HPV 16/18/45 Neg	Other HR HPV Pos	12.7 (7/55) (6.2, 20.5)	3.6 (2/55) (0.6, 9.1)
		Pos or Neg	HR HPV Pos	19.8 (17/86) (15.1, 23.9)	8.1 (7/86) (4.7, 10.3)
		HPV 16/18/45 Neg*	HR HPV Neg	1.2 (2/167) (0.2, 3.5)	0.6 (1/167) (0.0, 2.3)
			Prevalence	7.5% (19/253)	3.2% (8/253)
≥ 40 Years	Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	33.3 (5/15) (12.4, 55.0)	20.0 (3/15) (4.1, 36.0)
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	66.7 (4/6) (27.1, 93.5)	33.3 (2/6) (6.2, 69.2)
		HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	11.1 (1/9) (0.5, 39.7)	11.1 (1/9) (0.5, 37.1)
		HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	--- (0/0)	--- (0/0)
		HPV 16/18/45 Neg	Other HR HPV Pos	3.4 (1/29) (0.1, 14.0)	0 (0/29) (0.0, 8.2)
		Pos or Neg	HR HPV Pos	13.6 (6/44) (6.5, 20.6)	6.8 (3/44) (1.8, 11.4)
	Negative	HPV 16/18/45 Neg*	HR HPV Neg	1.9 (4/213) (0.6, 3.4)	0.9 (2/213) (0.1, 2.0)
			Prevalence	3.9% (10/257)	1.9% (5/257)

AHPV-GT = APTIMA HPV 16 18/45 Genotype Assay, HR = High-risk, Pos = Positive, Neg = Negative
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

The relative risk of disease for APTIMA HPV 16 18/45 Genotype Assay positive versus negative outcomes are shown in Table 5. Women who had HPV types 16, 18, and/or 45 present were 13.2 times more likely to have ≥CIN2 and 22.1 times more likely to have ≥CIN3 than women with no high-risk HPV types present. Women who had HPV types 16, 18, and/or 45 present were 2.0 times more likely to have ≥CIN2 and 3.8 times more likely to have ≥CIN3 than women with one or more of the other 11 high-risk HPV types present.

Table 5: ASC-US ≥ 21 Years Population: Relative Risk of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay

APTIMA Assay Result Interpretation*	≥CIN2	≥CIN3
	Relative Risk (95% CI)	Relative Risk (95% CI)
HPV 16 and/or 18/45 Positive vs HR HPV Negative	13.2 (7.0, 24.7)	22.1 (7.7, 63.8)
HPV 16 and/or 18/45 Positive vs Other HR HPV Positive	2.0 (1.3, 3.1)	3.8 (1.8, 7.8)
Other HR HPV Positive vs HR HPV Negative	6.5 (3.4, 12.3)	5.9 (1.9, 18.6)
HR HPV Positive vs HR HPV Negative	8.7 (4.8, 15.9)	11.4 (4.0, 32.0)
Prevalence	8.8% (79/893)	3.7% (33/893)

CI = Confidence Interval, HR = High-risk
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

The likelihood ratios (≥CIN2 and ≥CIN3) by the APTIMA HPV 16 18/45 Genotype Assay result are shown in Table 6. HPV types 16, 18, and/or 45 were 4.2 times more likely to be present in a woman with ≥CIN2 and 5.1 times more likely to be present in a woman with ≥CIN3.

Table 6: ASC-US ≥ 21 Years Population: Likelihood Ratios for ≥CIN2 and ≥CIN3 by Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay

APTIMA Assay Result Interpretation*	≥CIN2	≥CIN3
	Likelihood Ratio (95% CI)	Likelihood Ratio (95% CI)
HPV 16 and/or 18/45 Positive	4.2 (3.0, 5.8)	5.1 (3.4, 6.9)
Other HR HPV Positive	1.7 (1.3, 2.3)	1.2 (0.6, 1.9)
HR HPV Negative	0.2 (0.1, 0.4)	0.2 (0.1, 0.4)

CI = Confidence Interval, HR = High-risk
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

NILM ≥ 30 Years Population: APTIMA HPV 16 18/45 Genotype Assay Clinical Performance with ThinPrep Liquid Cytology Specimens

In total, there were 540 evaluable women 30 years of age and older with NILM cytology results and APTIMA HPV Assay positive results whose referral cytology samples were eligible for testing with the APTIMA HPV 16 18/45 Genotype Assay. Of these, 25 women (18 attended colposcopy and 7 did not attend colposcopy) did not have referral cytology sample volume available for test18

ing in this study; after a missing values analysis, they were not included in the performance calculations. The 515 evaluable women had valid APTIMA HPV 16 18/45 Genotype Assay results. Of these, 317 attended colposcopy. Fifteen (15) women had ≥CIN2 and 10 had ≥CIN3; 283 women had normal/CIN1 histology; 19 women had undetermined disease status.

Of the 298 evaluable women with conclusive disease status and APTIMA HPV Assay positive results, 61 had APTIMA HPV 16 18/45 Genotype Assay positive results, indicating the presence of HPV 16 and/or HPV 18/45; 237 had negative results, indicating the presence of one or more of the other 11 high-risk HPV types. An additional 505 evaluable women 30 years of age and older with NILM cytology results and conclusive disease status had APTIMA HPV Assay negative results during the CLEAR trial. An APTIMA HPV Assay negative result indicates that none of the 14 high-risk HPV types are present and were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis. The results of the APTIMA HPV 16 18/45 Genotype Assay by APTIMA HPV Assay result and consensus histology review panel diagnosis are presented in Table 7.

Table 7: NILM ≥ 30 Years Population: Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay by Consensus Histology Review Panel Diagnosis

APTIMA HPV Assay Result	AHPV-GT Assay Result*	Interpretation	Consensus Histology Review Panel Diagnosis						
			Undetermined**	Normal	CIN1	CIN2	CIN3	Cancer	Total
Positive	HPV 16 Neg, HPV 18/45 Neg	Other HR HPV Pos	16	218	11	4	4	0	253
	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos	2	27	0	0	3	1	33
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos	1	26	1	1	0	2	31
	HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	0	0	0	0	0	0	0
		Total	19	271	12	5	7	3	317
Negative	HPV 16/18/45 Neg***	HR HPV Neg	25	483	17	4	1	0	530
		Total	44	754	29	9	8	3****	847

AHPV-GT = APTIMA HPV 16 18/45 Genotype Assay, HR = High-risk, Pos = Positive, Neg = Negative
*All samples had final valid results (upon initial testing or after resolution of initial invalids per procedure).
**44 women attended the colposcopy visit but a diagnosis could not be determined for the following reasons: consensus could not be reached due to inadequate specimens (n=28), no biopsies collected due to underlying factors (n=13), no biopsies collected or reviewed due to error (n=3).
***Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.
****Three women had adenocarcinoma in situ (AIS).

Of the 515 women with APTIMA HPV Assay positive results and APTIMA HPV 16 18/45 Genotype Assay results, 217 women had unverified (including undetermined) disease status (Table 8). Of the 10,331 women with APTIMA HPV Assay negative results from the original CLEAR trial, 9,826 had unverified disease status. Because the study was designed such that only randomly selected women with negative results for both the APTIMA HPV Assay and the commercially available HPV DNA test were referred to colposcopy, the proportion of women with unverified disease status was high in this group (96.6%). To adjust for this verification bias, a multiple imputation method was used to estimate the number of women with disease that would have been identified if all women had undergone colposcopy. Both verification-bias adjusted performance estimates and unadjusted performance estimates based on the 803 women with verified disease status are presented.

Table 8: NILM ≥ 30 Years Population: Classification of Evaluable NILM Women by APTIMA HPV Assay, APTIMA HPV 16 18/45 Genotype Assay, HPV DNA Test Results, Disease Status (≥CIN2 and ≥CIN3), and Disease Verification Status

APTIMA HPV Assay Result*	AHPV-GT Assay Result*	HPV DNA Test	Total Women	Verified Disease Status: ≥CIN2		Verified Disease Status: ≥CIN3		Unverified Disease Status
				Diseased Women (≥CIN2)	Non-Diseased Women (<CIN2)	Diseased Women (≥CIN3)	Non-Diseased Women (<CIN3)	Women with Unknown Disease Status (% Unknown)
Positive	Positive	Positive	83	6	48	5	49	29 (34.9%)
	Positive	Negative	9	1	5	1	5	3 (33.3%)
	Positive	No Result**	2	0	1	0	1	1 (50.0%)
	Negative	Positive	271	7	171	4	174	93 (34.3%)
	Negative	Negative	137	1	52	0	53	84 (61.3%)
	Negative	No Result**	13	0	6	0	6	7 (53.8%)
		Total	515	15	283	10	288	217 (42.1%)
Negative	N/A***	Positive	306	3	178	1	180	125 (40.8%)
	N/A***	Negative	9,420	1	322	0	323	9,097 (96.6%)
	N/A***	No Result**	605	1	0	0	1	604 (99.8%)
		Total	10,846	20	783	11	792	10,043 (92.6%)

AHPV-GT = APTIMA HPV 16 18/45 Genotype Assay, N/A = Not Applicable
*All samples had final valid results (upon initial testing or after resolution of initial invalids per procedure).
**620 women with APTIMA HPV Assay results did not have HPV DNA Test results primarily due to insufficient volume of the cytology specimen.
***Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

The adjusted absolute risks of disease (≥CIN2 and ≥CIN3) by APTIMA HPV 16 18/45 Genotype Assay result and APTIMA HPV Assay result are shown in Table 9a. The risk of ≥CIN2 in women with HPV types 16, 18, and/or 45 present was 12.6% compared to 3.4% in women with one or more of the other 11 high-risk HPV types present and 0.6% in women with no high-risk HPV types present. The unadjusted absolute risks of disease are shown overall in Table 9b and by age group in Table 10.

Table 9a: NILM ≥ 30 Years Population: Absolute Risk of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay (Verification-Bias Adjusted Estimates)

APTIMA HPV Assay Result	AHPV-GT Assay Result	Interpretation	≥CIN2	≥CIN3
			Absolute Risk (95% CI)	Absolute Risk (95% CI)
Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	12.6 (3.7, 21.4)	9.5 (2.1, 16.8)
	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	14.5 (2.1, 26.9)	12.1 (0.7, 23.4)
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	10.7 (0.0, 22.5)	6.9 (0.0, 16.2)
	HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	N/A	N/A
	HPV 16/18/45 Neg	Other HR HPV Pos	3.4 (1.2, 5.6)	1.8 (0.1, 3.5)
	Pos or Neg	HR HPV Pos	5.0 (2.6, 7.5)	3.2 (1.3, 5.2)
Negative	HPV 16/18/45 Neg*	HR HPV Neg	0.6 (0.1, 1.2)	0.4 (0.0, 0.7)
		Prevalence	0.9%	0.5%

AHPV-GT = APTIMA HPV 16 18/45 Genotype Assay, HR = High-risk, Pos = Positive, Neg = Negative, N/A = Not Applicable
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

Table 9b: NILM ≥ 30 Years Population: Absolute Risk of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay (Unadjusted Estimates)

APTIMA HPV Assay Result	AHPV-GT Assay Result	Interpretation	≥CIN2	≥CIN3
			Absolute Risk (95% CI)	Absolute Risk (95% CI)
Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	11.5 (7/61) (5.4, 18.9)	9.8 (6/61) (4.6, 15.2)
	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	12.9 (4/31) (4.0, 26.0)	12.9 (4/31) (4.3, 23.8)
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	10.0 (3/30) (2.4, 23.0)	6.7 (2/30) (0.8, 17.7)
	HPV 16 Neg, HPV 18/45 Pos	HPV 16 & 18/45 Pos	N/A (0/0)	N/A (0/0)
	HPV 16/18/45 Neg	Other HR HPV Pos	3.4 (8/237) (1.7, 5.3)	1.7 (4/237) (0.6, 3.2)
	Pos or Neg	HR HPV Pos	5.0 (15/298) (3.6, 6.2)	3.4 (10/298) (2.3, 3.9)
Negative	HPV 16/18/45 Neg*	HR HPV Neg	1.0 (5/505) (0.4, 1.9)	0.2 (1/505) (0.0, 0.9)
		Prevalence	2.5% (20/803)	1.4% (11/803)

AHPV-GT = APTIMA HPV 16 18/45 Genotype Assay, HR = High-risk, Pos = Positive, Neg = Negative, N/A = Not Applicable
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

Table 10: NILM ≥ 30 Years Population: Absolute Risk of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay by Age Group (Unadjusted Estimates)

	APTIMA HPV Assay Result	AHPV-GT Assay Result	Interpretation	≥CIN2	≥CIN3
				Absolute Risk (95% CI)	Absolute Risk (95% CI)
30 to 39 Years	Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	8.8 (3/34) (2.2, 17.8)	5.9 (2/34) (1.0, 13.3)
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	0 (0/17) (0.0, 15.5)	0 (0/17) (0.0, 14.3)
		HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	17.6 (3/17) (3.2, 35.4)	11.8 (2/17) (1.3, 27.0)
		HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	N/A (0/0)	N/A (0/0)
		HPV 16/18/45 Neg	Other HR HPV Pos	4.0 (5/124) (1.7, 6.2)	2.4 (3/124) (0.7, 4.2)
		Pos or Neg	HR HPV Pos	5.1 (8/158) (3.2, 6.1)	3.2 (5/158) (1.5, 4.0)
	Negative	HPV 16/18/45 Neg*	HR HPV Neg	0.5 (1/217) (0.0, 1.9)	0.5 (1/217) (0.0, 1.7)
			Prevalence	2.4% (9/375)	1.6% (6/375)
≥ 40 Years	Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	14.8 (4/27) (4.7, 27.3)	14.8 (4/27) (5.1, 22.8)
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	28.6 (4/14) (6.3, 50.7)	28.6 (4/14) (6.4, 46.5)
		HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	0 (0/13) (0.0, 20.1)	0 (0/13) (0.0, 17.1)
		HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	N/A (0/0)	N/A (0/0)
		HPV 16/18/45 Neg	Other HR HPV Pos	2.7 (3/113) (0.7, 5.8)	0.9 (1/113) (0.0, 3.1)
		Pos or Neg	HR HPV Pos	5.0 (7/140) (2.6, 7.0)	3.6 (5/140) (1.9, 4.2)
	Negative	HPV 16/18/45 Neg*	HR HPV Neg	1.4 (4/288) (0.5, 2.5)	0 (0/288) (0.0, 0.8)
			Prevalence	2.6% (11/428)	1.2% (5/428)

AHPV-GT = APTIMA HPV 16 18/45 Genotype Assay, HR = High-risk, Pos = Positive, Neg = Negative, N/A = Not Applicable
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

The relative risk of disease for APTIMA 16 18/45 Genotype Assay positive versus negative outcomes are shown in Table 11 (verification-bias adjusted) and Table 12 (unadjusted). Women who had HPV types 16, 18, and/or 45 present were 20.9 times more likely to have ≥CIN2 and 29.4 times more likely to have ≥CIN3 than women with no high-risk HPV types present. Women who had HPV types 16, 18, and/or 45 present were 3.7 times more likely to have ≥CIN2 and 5.3 times more likely to have ≥CIN3 than women with one or more of the other 11 high-risk HPV types present.

Table 11: NILM ≥ 30 Years Population: Relative Risk of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay (Verification-Bias Adjusted Estimates)

APTIMA Assay Test Interpretation*	≥CIN2	≥CIN3
	Relative Risk (95% CI)	Relative Risk (95% CI)
HPV 16 and/or 18/45 Pos vs HR HPV Neg	20.9 (6.3, 69.3)	29.4 (7.2, 120.8)
HPV 16 and/or 18/45 Pos vs Other HR HPV Pos	3.7 (1.5, 9.5)	5.3 (1.5, 18.2)
Other HR HPV Pos vs HR HPV Neg	5.6 (1.8, 17.7)	5.6 (1.2, 26.0)
HR HPV Pos vs HR HPV Neg	8.5 (2.9, 24.8)	10.1 (2.7, 38.2)
Prevalence	0.9%	0.5%

CI = Confidence Interval, HR = High-risk, Pos = Positive, Neg = Negative
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

Table 12: NILM ≥ 30 Years Population: Relative Risk of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay (Unadjusted Estimates)

APTIMA Assay Test Interpretation*	≥CIN2	≥CIN3
	Relative Risk (95% CI)	Relative Risk (95% CI)
HPV 16 and/or 18/45 Pos vs HR HPV Neg	11.6 (3.8, 35.4)	49.7 (6.1, 406)
HPV 16 and/or 18/45 Pos vs Other HR HPV Pos	3.4 (1.3, 9.0)	5.8 (1.7, 20.0)
Other HR HPV Pos vs HR HPV Neg	3.4 (1.1, 10.3)	8.5 (1.0, 75.8)
HR HPV Pos vs HR HPV Neg	5.1 (1.9, 13.8)	16.9 (2.2, 132)
Prevalence	2.5% (20/803)	1.4% (11/803)

CI = Confidence Interval, HR = High-risk, Pos = Positive, Neg = Negative
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

The likelihood ratios (≥CIN2 and ≥CIN3) by the APTIMA 16 18/45 Genotype Assay result are shown in Table 13 (verification-bias adjusted) and Table 14 (unadjusted). HPV types 16, 18, and/or 45 were 17.1 times more likely to be present in a woman with ≥CIN2 and 21.9 times more likely to be present in a woman with ≥CIN3.

Table 13: NILM ≥ 30 Years Population: Likelihood Ratios for ≥CIN2 and ≥CIN3 by Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay (Verification-Bias Adjusted Estimates)

APTIMA Assay Result Interpretation	≥CIN2	≥CIN3
	Likelihood Ratio (95% CI)	Likelihood Ratio (95% CI)
HPV 16 and/or 18/45 Positive	17.1 (6.2, 46.9)	21.9 (7.3, 65.2)
Other HR HPV Positive	4.2 (1.7, 10.1)	3.8 (1.2, 12.6)
HR HPV Negative	0.7 (0.5, 1.0)	0.7 (0.4, 1.1)

CI = Confidence Interval, HR = High-risk
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

Table 14: NILM ≥ 30 Years Population: Likelihood Ratios for ≥CIN2 and ≥CIN3 by Results of the APTIMA HPV 16 18/45 Genotype Assay and APTIMA HPV Assay (Unadjusted Estimates)

APTIMA Assay Result Interpretation*	≥CIN2	≥CIN3
	Likelihood Ratio (95% CI)	Likelihood Ratio (95% CI)
HPV 16 and/or 18/45 Positive	5.1 (2.3, 9.1)	7.9 (3.5, 12.9)
Other HR HPV Positive	1.4 (0.7, 2.2)	1.2 (0.4, 2.3)
HR HPV Negative	0.4 (0.1, 0.7)	0.1 (0.0, 0.6)

CI = Confidence Interval, HR = High-risk
*Women with APTIMA HPV Assay negative results were designated as APTIMA HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

APTIMA HPV 16 18/45 Genotype Assay Clinical Performance with Cervical Specimen Collection and Transport Specimens

The APTIMA HPV 16 18/45 Genotype Assay performance was evaluated using CSCT samples (n=566) collected from women at routine screening or follow-up visits. Specimens were collected from women in Canada, Mexico and Germany and initially tested with the APTIMA HPV Assay. Residual specimens were tested with the APTIMA HPV 16 18/45 Genoytpe Assay.

Clinical agreement of the APTIMA HPV 16 18/45 Genotype Assay for detection of high-risk HPV 16, 18 and 45 was determined. HPV 16, 18 and 45 status was based on results of the following reference methods: 1) APTIMA HPV Assay negative specimens with a negative high-risk HPV DNA test result were classified as 16, 18 and 45 negative; 2) APTIMA HPV Assay positive specimens were genotyped using a commercially available HPV DNA genotyping test and a validated RT-PCR sequencing test (to resolve APTIMA HPV 16 18/45 Genotype Assay and DNA genotype test discordants); specimens with HPV 16, 18 or 45 were classified as positive, and those without were classified as negative. Positive and negative percent agreements and associated 95% Score confidence intervals were calculated. Results are presented in Table 15.

Table 15: Clinical Agreement of the APTIMA HPV 16 18/45 Genotype Assay on the TIGRIS DTS System for Detection of High-risk HPV 16, 18 and 45 in APTIMA CSCT specimens

		Reference Method				
		16 Pos 18/45 Neg	**16 Neg 18/45 Pos**	**16 Pos 18/45 Pos**	**16 Neg 18/45 Neg**	**Total**
APTIMA HPV 16 18/45 Genotype Assay	**16 Pos 18/45 Neg**	125	0	1	0	126
	16 Neg 18/45 Pos	0	43	0	1	44
	16 Pos 18/45 Pos	0	0	8	1	9
	16 Neg 18/45 Neg	1	1	0	197	199
	Total	126	44	9	199	378

Positive agreement: 98.3% (176/179) (95% CI: 95.2, 99.4)

Negative agreement: 99.0% (197/199) (95% CI: 96.4, 99.7)

Analytical Sensitivity

The Limit of Detection (LOD) at the clinical cutoff is a concentration that is positive (above the clinical cutoff) 95% of the time. The LOD of the APTIMA HPV 16 18/45 Genotype Assay was estimated by testing individual negative clinical ThinPrep liquid cytology specimens spiked with HPV *in vitro* transcripts at various concentrations. Thirty replicates of each copy level were tested with each of three reagent lots for a total of 90 replicates. Testing was performed over 6 days, with 3 runs performed per day and 5 replicates of a given genotype tested in each run. The 95% detection limit (Table 16) was calculated from Probit regression analysis of the positivity results for each dilutional panel.

Table 16: Limit of Detection at the Clinical Cutoff of the APTIMA HPV 16 18/45 Genotype Assay

Target	Limit of Detection copies/reaction (95% CI)
HPV 16	57.3 (46.5 - 74.6)
HPV 18	84.8 (66.1 - 115.6)
HPV 45	60.0 (46.6 - 82.3)

Positive agreement: 98.3% (176/179) (98.3% CI: 95.2, 99.4)

Negative agreement: 99.5% (385/387) (99.0% CI: 96.4, 99.7)

Assay Precision

APTIMA HPV 16 18/45 Genotype Assay precision was evaluated in two studies using the same 22-member panel. Study 1 was conducted at 3 external testing sites to determine assay reproducibility. Study 2 was conducted in-house to measure assay repeatability. The panel included 14 HPV 16 and/or 18/45-positive members with concentrations at or above the limit of detection of the assay (expected positivity: ≥ 95%), 5 HPV 16 and/or 18/45-positive members with concentrations below the limit of detection of the assay (expected positivity: >0% to <25%), and 3 HPV-negative members. HPV 16 and/or 18/45-positive panel members were prepared by spiking HPV-infected cultured cells (SiHa, HeLa, and MS751; ATCC, Manassas, Virginia) into pooled residual ThinPrep liquid cytology specimens or diluting HPV 16, 18, and/or 45 clinical specimens into pooled residual ThinPrep liquid cytology specimens. HPV-negative panel members were prepared with pooled ThinPrep liquid cytology specimens.

In Study 1, 2 operators at each of the 3 testing sites (1 instrument per site) performed 2 APTIMA HPV 16 18/45 Genotype Assay worklists per day over 3 days. Testing was performed using 1 reagent lot. Each worklist contained 3 replicates of each of the reproducibility panel members. One hundred eight (108) individual sample tubes were tested for each panel member (3 sites x 1 instrument x 2 operators x 1 lot x 2 worklists per day x 3 days x 3 replicates). In Study 2, testing was conducted in-house over 20 days with a total of 162 reactions tested for each panel member (1 site x 3 instruments x 3 operators x 3 lots x 2 worklists x 3 replicates).

The panel members are described in Table 17, along with a summary of the agreement with expected results.

Table 17: APTIMA HPV 16 18/45 Genotype Assay Precision Study 1 and 2: Panel Description and Percent Agreement With Results

Panel Description (cells/reaction)	Expected Result		Percent Agreement (95% CI)	
	HPV 16	HPV 18/45	Study 1 (3 testing sites)	Study 2 (1 testing site)
SiHa cells (3.0 cells)	Pos	Neg	100 (108/108) (96.6, 100)	98.8 (160/162) (95.6, 99.7)
HeLa cells (0.6 cells)	Neg	Pos	93.5 (101/108) (87.2, 96.8)	98.1 (159/162) (94.7, 99.4)
MS751 cells (11.0 cells)	Neg	Pos	92.6 (100/108) (86.1, 96.2)	92.6 (150/162) (87.5, 95.7)
HPV 16 clinical sample	Pos	Neg	100 (107/107) (96.5, 100)	100 (162/162) (97.7, 100)
HPV 18/45 clinical sample	Neg	Pos	99.1 (107/108) (94.9, 99.8)	98.1 (159/162) (94.7, 99.4)
SiHa cells (1.6 cells) & HeLa cells (3.3 cells)	Pos	Pos	100 (108/108) (96.6, 100)	98.8 (160/162) (95.5, 99.7)
SiHa cells (1.6 cells) & MS751 cells (42.5 cells)	Pos	Pos	100 (108/108) (96.6, 100)	98.8 (160/162) (95.6, 99.7)
SiHa cells (15.7 cells) & HeLa cells (0.3 cells)	Pos	Pos	63.9 (69/108) (54.5, 72.3)	67.7 (109/161) (60.1, 74.4)
SiHa cells (15.7 cells) & MS751 cells (4.3 cells)	Pos	Pos	98.1 (106/108) (93.5, 99.5)	92 (149/162) (86.8, 95.3)
SiHa cells (1.6 cells)	Pos	Neg	97.2 (105/108) (92.1, 99.1)	98.1 (159/162) (94.7, 99.4)
HeLa cells (0.3 cells)	Neg	Pos	71.3 (77/108) (62.1, 79.0)	92.5 (149/161) (87.4, 95.7)
MS751 cells (4.3 cells)	Neg	Pos	86.1 (93/108) (78.3, 91.4)	69.1 (112/162) (61.6, 75.7)
HPV 16 clinical sample	Pos	Neg	97.2 (104/107) (92.1, 99.0)	93.8 (151/161) (88.9, 96.6)
HPV 18/45 clinical sample	Neg	Pos	88.0 (95/108) (80.5, 92.8)	79.6 (129/162) (72.8, 85.1)
SiHa cells (0.1 cells)	Neg	Neg	85.2 (92/108) (77.3, 90.7)	84.6 (137/162) (78.2, 89.3)
HeLa cells (0.02 cells)	Neg	Neg	92.6 (100/108) (86.1, 96.2)	86.4 (140/162) (80.3, 90.9)
MS751 cells (0.04 cells)	Neg	Neg	97.2 (105/108) (92.1, 99.1)	98.1 (159/162) (94.7, 99.4)
HPV 16 clinical sample	Neg	Neg	95.4 (103/108) (89.6, 98.0)	92 (149/162) (86.8, 95.3)
HPV 18/45 clinical sample	Neg	Neg	80.6 (87/108) (72.1, 86.9)	80.9 (131/162) (74.1, 86.2)
HPV-negative clinical sample 1	Neg	Neg	100 (108/108) (96.6, 100)	99.4 (161/162) (96.6, 99.9)
HPV-negative clinical sample 2	Neg	Neg	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV-negative clinical sample 3	Neg	Neg	100 (108/108) (96.6, 100)	99.4(161/162) (96.6, 99.9)

Pos = positive, Neg = negative, CI = Score Confidence Interval

Note: *The percent agreement may have been affected by variations in spiking, diluting, and/or aliquoting.*

Cross-Reactivity

The analytical specificity of the APTIMA HPV 16 18/45 Genotype Assay was evaluated with pools of residual ThinPrep liquid cytology specimens diluted 1:2.9 into STM (comparable to specimen transferred to an APTIMA Transfer tube) and spiked with cultured bacteria, yeast, or fungi; cultured virus; or non-targeted HPV *in vitro* transcripts. The organisms and test concentrations for which no cross reactivity was observed are identified in Table 18. The study criteria for assessing the effect of the presence of microorganism on the specificity of the assay were based on positivity.

Table 18: Analytical Specificity Panel: Organisms and Concentration with No Cross-Reactivity

Organism	Test Concentration with No Cross-Reactivity	Organism	Test Concentration with No Cross-Reactivity
Bacteria			
Acinetobacter lwoffii	$1x10^6$ CFU/mL	*Lactobacillus acidophilus*	$1x10^6$ CFU/mL
Actinomyces israelii	$1x10^6$ CFU/mL	*Lactobacillus crispatus*	$1x10^6$ CFU/mL
Alcaligenes faecalis	$1x10^6$ CFU/mL	*Listeria monocytogenes*	$1x10^6$ CFU/mL
Atopobium vaginae	$1x10^6$ CFU/mL	*Mobiluncus curtisii*	$1x10^6$ CFU/mL
Bacteroides fragilis	$1x10^6$ CFU/mL	*Mycoplasma genitalium**	$2.5x10^6$ copies/mL
Bifidobacterium adolescentis	$1x10^6$ CFU/mL	*Mycoplasma hominis*	$1x10^6$ CFU/mL
Campylobacter jejuni	$1x10^6$ CFU/mL	*Neisseria gonorrhoeae*	$1x10^6$ CFU/mL
Chlamydia trachomatis	$1x10^5$ IFU/mL	*Peptostreptococcus magnus*	$1x10^6$ CFU/mL
Clostridium difficile	$1x10^6$ CFU/mL	*Prevotaella bivia*	$1x10^6$ CFU/mL
Corynebacterium genitalium	$1x10^6$ CFU/mL	*Propionibacterium acnes*	$1x10^6$ CFU/mL
Cryptococcus neoformans	$1x10^6$ CFU/mL	*Proteus vulgaris*	$1x10^6$ CFU/mL
Enterobacter cloacae	$1x10^6$ CFU/mL	*Pseudomonas aeruginosa*	$1x10^6$ CFU/mL
Enterococcus faecalis	$1x10^6$ CFU/mL	*Staphylococcus aureus*	$1x10^6$ CFU/mL
Escherichia coli	$1x10^6$ CFU/mL	*Staphylococcus epidermidis*	$1x10^6$ CFU/mL
Fusobacterium nucleatum	$1x10^6$ CFU/mL	*Streptococcus agalactiae*	$1x10^6$ CFU/mL
Gardnerella vaginalis	$1x10^6$ CFU/mL	*Streptococcus pyogenes*	$1x10^6$ CFU/mL
Haemophilus ducreyi	$1x10^6$ CFU/mL	*Ureaplasma urealyticum*	$1x10^6$ CFU/mL
Klebsiella pneumoniae	$1x10^6$ CFU/mL		
Non-targeted High-risk HPV genotypes*			
HPV 31	$2.5x10^6$ copies/mL	HPV 56	$2.5x10^6$ copies/mL
HPV 33	$2.5x10^6$ copies/mL	HPV 58	$2.5x10^6$ copies/mL
HPV 35	$2.5x10^6$ copies/mL	HPV 59	$2.5x10^6$ copies/mL
HPV 39	$2.5x10^6$ copies/mL	HPV 66	$2.5x10^6$ copies/mL
HPV 51	$2.5x10^6$ copies/mL	HPV 68	$2.5x10^6$ copies/mL
HPV 52	$2.5x10^6$ copies/mL		

Table 18: Analytical Specificity Panel: Organisms and Concentration with No Cross-Reactivity *(continued)*

Organism	Test Concentration with No Cross-Reactivity	Organism	Test Concentration with No Cross-Reactivity
Viruses			
Adenovirus	$5.25x10^7$ PFU/mL	HIV-1	$2.5x10^6$ copies/mL
Cytomegalovirus	$1.58x10^6$ $TCID_{50}$/mL	Herpes simplex virus 1	$3.39x10^6$ $TCID_{50}$/mL
Epstein-Barr virus	$1.59x10^5TD_{50}$/mL	Herpes simplex virus 2	$2.29x10^6$ $TCID_{50}$/mL
Non-targeted other HPV genotypes*			
HPV 6	$2.5x10^6$ copies/mL	HPV 53	$2.5x10^6$ copies/mL
HPV 11	$2.5x10^6$ copies/mL	HPV 67	$2.5x10^6$ copies/mL
HPV 26	$2.5x10^6$ copies/mL	HPV 69	$2.5x10^6$ copies/mL
HPV 30	$2.5x10^6$ copies/mL	HPV 70	$2.5x10^6$ copies/mL
HPV 34	$2.5x10^6$ copies/mL	HPV 73	$2.5x10^6$ copies/mL
HPV 42	$2.5x10^6$ copies/mL	HPV 82	$2.5x10^6$ copies/mL
HPV 43	$2.5x10^6$ copies/mL	HPV 85	$2.5x10^6$ copies/mL
HPV 44	$2.5x10^6$ copies/mL		
Yeast/protozoa			
Candida albicans	$1x10^6$ CFU/mL	*Trichomonas vaginalis***	$1x10^5$ cells/mL

CFU = Colony Forming Units, PFU = Plaque Forming Units, TD_{50} = Transformation Dose 50, $TCID_{50}$ = Tissue Culture Infective Dose 50

**In vitro* transcript tested.

**Although no cross-reactivity was observed for *Trichomonas vaginalis*, interference was observed (see below).

The analytical sensitivity of the APTIMA HPV 16 18/45 Genotype Assay in the presence of microorganisms was evaluated with the same panel described in Table 18, which was also spiked with a low concentration of HPV infected SiHa cells (1.6 cells/reaction) and HPV infected HeLa cells (0.3 cells/reaction). The study criteria for assessing the effect of the presence of microorganism on the sensitivity of the assay were based on positivity. The presence of the microorganisms did not interfere with the APTIMA HPV 16 18/45 Genotype Assay with the exception of *Trichomonas vaginalis* (TV). Interference was observed with TV when present at concentrations greater than 3×10^4 cells/mL.

Interference

The substances described in Table 19 were individually spiked into pooled ThinPrep liquid cytology specimens diluted 1:2.9 in STM at the concentrations specified in the table. All substances were tested with the APTIMA HPV 16 18/45 Genotype Assay in the presence and absence of HPV infected cultured cells (SiHa, 1.6 cells/ reaction and HeLa, 0.3 cells/reaction). Interference was observed with the following when present at concentrations greater than those specified: vaginal lubricants (containing Polyquaternium 15) at 1% w/v, anti-fungal cream (containing tioconazole) at 0.03% w/v, mucus at 0.3% w/v, vaginal hormones (containing progesterone) at 1% w/v.

Table 19: Substances Tested for Possible Interference with the APTIMA HPV 16 18/45 Genotype Assay

Product Category	Product Brand or Type	Highest concentration tested that did not interfere with the assay*
Vaginal Lubricant	KY natural feeling liquid	10% v/v
	Up & up (Target brand) personal lubricant liquid	
	Astroglide**	1% w/v
Spermicide/Contraceptive Jelly	Vaginal Contraceptive Foam (VCF)	10% w/v
	Options Conceptrol Vaginal Contraceptive Gel	
Anti-fungal cream	Up & up (Target brand) miconazole 3	10% w/v
	Monistat 3 Combination Pack	
	Up & up (Target brand) Tioconazole 1	0.03% w/v
Douche	Summer's Eve Douche	10% v/v
	Up & up (Target brand) feminine douche	
Feminine Spray	Summer's Eve Feminine Deodorant Spray	10% w/v
	FDS Feminine Deodorant Spray	
Mucus	Porcine mucin	0.3% w/v
Intravaginal Hormones	Estrace Vaginal Cream (estrogen)	10% w/v
	Crinone Cream (progesterone)	1% w/v
Whole Blood* **	whole blood	5% v/v
Leukocytes	leukocytes	$1x10^7$ cells/mL
Glacial Acetic Acid Wash Solution** **	Glacial Acetic Acid + CytoLyt Solution	2.6% v/v

*concentration in the test sample; ThinPrep liquid cytology specimen diluted 1:2.9 into STM (comparable to specimen transferred to an APTIMA Transfer tube)

**personal lubricants that contain Polyquaternium 15

***whole blood interfered with the assay when present at a 10% v/v test concentration

****glacial acetic acid wash solution prepared by mixing 1 part glacial acetic acid and 9 part Cytolyt solution as denoted in the ThinPrep 2000 System Operator's Manual.

Bibliography

1. **Walboomers, J. M., M.V. Jacobs, M.M. Manos, F.X. Bosch, J.A. Kummer, K.V. Shah, P.J. Snijders, J. Peto, C. J. Meijer, N. Muñoz.**1999. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 189:12-19.
2. **Li N., Franceschi S., Howell-Jones R., Snijders P.J.F., Clifford G.M.** Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. Int J Cancer. 2011;128: 927-935. doi 10.1002/ijc.25396
3. **Czegledy J., C. Losif, B.G. Hansson, M. Evander, L. Gergely, and G. Wadell.** 1995. Can a test for E6/E7 transcripts of human papillomavirus type 16 serve as a diagnostic tool for the detection of micrometastasis in cervical cancer? Int J Cancer. 64(3):211-5.
4. **Doorbar, J.** 2006. Molecular biology of human papillomavirus infection and cervical cancer. Clin Sci (Lond). 110(5):525-41.
5. **Burd, E.M.** 2003. Human papillomavirus and cervical cancer. Clin Microbiol Rev. 16(1):1-17.
6. **Lambert P.F., H. Pan, H.C. Pitot, A. Liem, M. Jackson, and A.E. Griep.** 1993. Epidermal cancer associated with expression of human papillomavirus type 16 E6 and E7 oncogenes in the skin of transgenic mice. Proc Natl Acad Sci U S A. 90(12):5583-7.
7. **Kjaer S.K., A.J.C. van den Brule, G., Paull, E.I. Svare, M.E. Sherman, B.L. Thomsen, M. Suntum, J.E. Bock, P.A. Poll, and C.J.L.M. Meijer.** 2002. Type specific persistence of high risk human papillomavirus (HPV) as indicator of high grade cervical squamous intraepithelial lesions in young women: population based prospective follow up study. BMJ. 325(7364): 572-579.
8. **Monsonego J., F.X. Bosch, P. Coursaget, J.T. Cox, E. Franco, I. Frazer, R. Sankaranarayanan, J. Schiller, A. Singer, T.C. Wright Jr, W. Kinney, C.J. Meijer, J. Linder, E. McGoogan, and C. Meijer.** 2004. Cervical cancer control, priorities and new directions. Int J Cancer. 108(3):329-33. Erratum in: Int J Cancer. 108(6):945.
9. **Cuschieri, K.S., M.J. Whitley, H.A. Cubie.** 2004. Human papillomavirus type specific DNA and RNA persistence--implications for cervical disease progression and monitoring. J. Med. Virol. 73(1): 65-70.
10. **De Sanjose S., et al.** 2010. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. The Lancet. DOI 10.1016/S1470-2045(10)70230-8.
11. **Burger R.A., B. J. Monk, T. Kurosaki, H. Anton-Culver, S. Vasilv, M. L. Berman and S.P. Wilczynski.** 1996. Human Papillomavirus Type 18: Association with poor prognosis in early stage cervical cancer. J. Nat. Cancer institute. 88(19): 1361-1368.
12. **Safaeian M., M. Schiffman, J. Gage, D. Solomon, C. Wheeler and P. Castle.** 2009. Detection of Precancerous Cervical Lesions Is Differential by Human Papillomavirus Type. Cancer Res. 69(8): 3262-3266.
13. **Khan, M.J., P.E. Castle, A.T. Lorincz, S. Wacholder, M. Sherman, D.R. Scott, B.B. Rush, A.G. Glass and M. Schiffman.** 2005. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. J. Natl. Cancer Inst. 97(14): 1072-1079.
14. **ASCCP: American Society for Colposcopy and Cervical Pathology.** HPV Genotyping Clinical Update. 2009. http://www.asccp.org/Portals/9/docs/pdfs/Consensus%20Guidelines/clinical_update_20090408.pdf. Accessed March 22, 2012.
15. **Wright T.C., S. Massad, C. J. Dunton, M. Spitzer, E.J. Wilkinson and D. Solomon.** 2007. 2006 Consensus guidelines for the management of women with abnormal cervical screening tests. Journal of Lower Genital Tract Disease. 11(4):201-222.
16. **Kacian, D.L. and T.J. Fultz.** 1995. Nucleic acid sequence amplification methods. U. S. Patent 5,399,491.
17. **Arnold, L. J., P. W. Hammond, W. A. Wiese, and N. C. Nelson.** 1989. Assay formats involving acridinium-ester-labeled DNA probes. Clin Chem. 35: 1588-1594.
18. **Nelson, N.C., A. BenCheikh, E. Matsuda, and M. Becker.** 1996. Simultaneous detection of multiple nucleic acid targets in a homogeneous format. Biochem. 35:8429-8438.
19. **Datta, S. D., L. A. Koutsky, S. Ratelle, E. R. Unger, J. Shlay, T. McClain, B. Weaver, P. Kerndt, J. Zenilman, M. Hagensee, C. J. Suhr, and H. Weinstock.** 2008. Human Papillomavirus Infection and Cervical Cytology in Women Screened for Cervical Cancer in the United States, 2003–2005. Annals Int Med. **148**:493.
20. **Clifford, G.M., S. Gallus, R. Herrero, N. Muñoz, P. J. F. Snijders, S. Vaccarella, P. T. H. Anh, C. Ferreccio, N. T. Hieu, E. Matos, M. Molano, R. Rajkumar, G. Ronco, S. de Sanjosé, H. R. Shin, S. Sukvirach, J. O. Thomas, S. Tunsakul, C. J. L. M. Meijer, S. Franceschi, and the IARC HPV Prevalence Surveys Study Group.** Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled Analysis. 2005. The Lancet. **366**, 991.

Gen-Probe Incorporated
San Diego, CA 92121

U.S. and international contact information:
Customer Service: +1 858 410 8002
customerservice@gen-probe.com
Technical Support: +1 858 410 8511
technicalsupport@gen-probe.com

Toll-free from U.S. and Canada:
Customer Service: +1 800 523 5001
Technical Support: +1 888 484 4747

www.gen-probe.com

This product is intended for use only in the field of human *in vitro* diagnostics.

503741 Rev. A

2013-03