Aptima™ HPV 16 18/45 Genotype Assay (Panther™System)

Instructions for Use
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) of human papillomavirus (HPV) 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 between HPV 18 and HPV 45.

The Aptima HPV 16 18/45 genotype assay can be used to test the following specimen types on the Panther System: cervical specimens collected in ThinPrep™ Pap Test vials containing PreservCyt™ solution pre- or post-Pap processing, cervical specimens collected with the Aptima Cervical Specimen Collection and Transport Kit, or cervical specimens collected in SurePath Preservative Fluid.

Aptima HPV 16 18/45 genotype assay is indicated for use for routine cervical cancer screening. Women who test positive or negative for HPV types 16, 18 or 45 should be triaged/followed-up in accordance with professional medical guidelines, the healthcare provider's assessment of screening, medical history, and other risk factors to assess the risk of cervical dysplasia and cancer.

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.[1]

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.[1,4]

Fourteen HPV genotypes are considered pathogenic or high-risk for the progression of cervical disease.[5] Multiple studies have linked genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 to disease progression.[2,6,7] Women with a persistent infection with one of these types have an increased risk for developing severe cervical dysplasia or cervical carcinoma.[5,8]

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.[7,10] 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.[7,10] 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).

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).

Summary of Safety and Performance

The SSP (Summary of Safety and Performance) is available in the European database on medical devices (Eudamed), where it is linked to the device identifiers (Basic UDI-DI). To locate the SSP for Aptima HPV 16 18/45 genotype assay, refer to the Basic Unique Device Identifier (BUDI): **54200455DIAGAPTHPVGTVK**.

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. For professional use.

C. For additional specific warnings and precautions related to instrumentation refer to the *Panther/Panther Fusion System Operator's Manual*.

Laboratory Related

D. Use only supplied or specified disposable laboratory ware.

E. 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.

F. **Warning: Irritant and Corrosive:** Avoid contact of Auto Detect 2 with skin, eyes and mucous membranes. If this fluid comes into contact with skin or eyes, wash the affected area with water. If this fluid spills, dilute the spill with water before wiping it dry.

G. 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 the *Panther System Test Procedure* for more information.

Specimen Related

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

I. 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.

J. 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.

K. 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.

L. Liquid can discharge from tube caps upon piercing under certain conditions. Refer to the *Panther System Test Procedure* for more information.

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

N. SurePath liquid cytology specimens should be rejected if a collection device is not present in the vial.

Assay Related

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

P. Avoid microbial and ribonuclease contamination of reagents.

Q. Do not use kit after its expiration date.

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

S. Aptima Assay Fluids and Auto Detect Reagents are not part of the Master Lot; any lot may be used.

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

U. Tips with hydrophobic plugs must be used.

V. Some reagents of this kit are labeled with risk and safety symbols.

Note: *Hazard Communication reflects the EU Safety Data Sheets (SDS) classifications. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com. For more information on the symbols, refer to the symbol legend on www.hologic.com/package-inserts.*

EU Hazard Information	
	**Selection Reagent** ***BORIC ACID 1 – 5%*** ***WARNING*** H315 – Causes skin irritation
—	**Target Capture Reagent** ***HEPES 5 – 10%*** ***EDTA 1 – 5%*** ***LITHIUM HYDROXIDE, MONOHYDRATE 1 - 5%*** — H412 – Harmful to aquatic life with long lasting effects P273 – Avoid release to the environment P280 – Wear eye protection/ face protection
—	**Amplification Reagent** *HEPES 25 – 30%* — H412 – Harmful to aquatic life with long lasting effects P273 – Avoid release to the environment P280 – Wear eye protection/ face protection
—	**Enzyme Reagent** *HEPES 1 – 5%* — H412 – Harmful to aquatic life with long lasting effects P273 – Avoid release to the environment P280 – Wear eye protection/ face protection
—	**Probe Reagent** *LAURYL SULFATE LITHIUM SALT 35 – 40%* *SUCCINIC ACID 10 – 15%* *LITHIUM HYDROXIDE, MONOHYDRATE 10 – 15%* — H412 – Harmful to aquatic life with long lasting effects P273 – Avoid release to the environment P280 – Wear eye protection/ face protection

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

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 72 hours when stored on-board the Panther 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 5000 Processor, ThinPrep 5000 Processor with Autoloader, or ThinPrep Genesis Processor, transfer 1 mL of the ThinPrep liquid cytology specimen into an Aptima Specimen Transfer tube, according to the Aptima Specimen Transfer Kit package insert.

SurePath liquid cytology specimens

1. Collect a SurePath liquid cytology specimen according to the SurePath Pap Test and/or PrepStain System instructions for use.

2. Transfer the SurePath liquid cytology specimen 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 CSCT Kit instructions for use.

B. Transport and storage before testing

ThinPrep liquid cytology specimens

1. Transport 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 to -70°C for up to 24 months.

SurePath liquid cytology specimens

1. Transport the SurePath liquid cytology specimens at 2°C to 25°C.
2. Specimens should be transferred to an Aptima Specimen Transfer tube within 7 days of collection.
3. Prior to transfer, SurePath liquid cytology specimens should be stored at 2°C to 25°C.
4. SurePath liquid cytology specimens transferred to an Aptima Specimen Transfer tube may be stored at 2°C to 25°C for up to 7 days.
5. Transferred SurePath specimens must be treated with the Aptima Transfer Solution prior to testing with the Aptima HPV 16 18/45 genotype assay. Treated samples may be stored at 2°C to 8°C for up to 17 days prior to testing with the Aptima HPV 16 18/45 genotype assay. Refer to the Specimen Transfer kit package insert for further details.

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 to -70°C 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.*

Panther System

Reagents and Materials Provided

Aptima HPV 16 18/45 Genotype Assay, 100 tests, (3 boxes) Cat. No. 303236

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** *Buffered solution containing solid-phase and capture oligomers(< 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 Hologic have catalog numbers listed, unless otherwise specified.*

	Cat. No.
Panther System	303095
Panther Run Kit	303096
Aptima Assay Fluids Kit (Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)	*303014*
Aptima Auto Detect Kit	*303013*
Multi-tube units (MTUs)	*104772-02*
Panther Waste Bag Kit	*902731*
Panther Waste Bin Cover	*504405*
Tips, 1000 µL filtered, conductive, liquid sensing, and disposable. *Not all products are available in all regions. Contact your representative for region-specific information*	901121 (10612513 Tecan) 903031 (10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128
Aptima Specimen Transfer Kit	301154C
Aptima Specimen Transfer Kit — printable	PRD-05110
Aptima Cervical Specimen Collection and Transport Kit	302657
Aptima Penetrable Caps	105668
Replacement non-penetrable caps	103036A
Spare Caps for 100 test kits:	
Amplification Reagent and Probe Reagent reconstitution solutions	*CL0041*
Enzyme Reagent reconstitution solution	*CL0041*
TCR and Selection Reagent	*501604*
Bleach, 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution	—
Disposable powderless gloves	—
Plastic-back laboratory bench covers	—
Lint-free wipes	—
Pipettor	—
Aptima Transfer Solution kit (for SurePath specimens only)	303658

Optional Materials

	Cat. No.
Bleach Enhancer for Cleaning	302101

Panther System Test Procedure

Note: *See the Panther/Panther Fusion System Operator's Manual for additional Panther System procedural information.*

A. Work Area Preparation

Clean work surfaces where reagents and samples will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces 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 and samples 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 Panther 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 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 (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 bottle to mix thoroughly. Avoid creating foam while swirling the bottle (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 glass vial (Figure 1, Step 6).
   j. Recap the plastic bottle. Record operator initials and the reconstitution date on the label (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 Panther 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.*

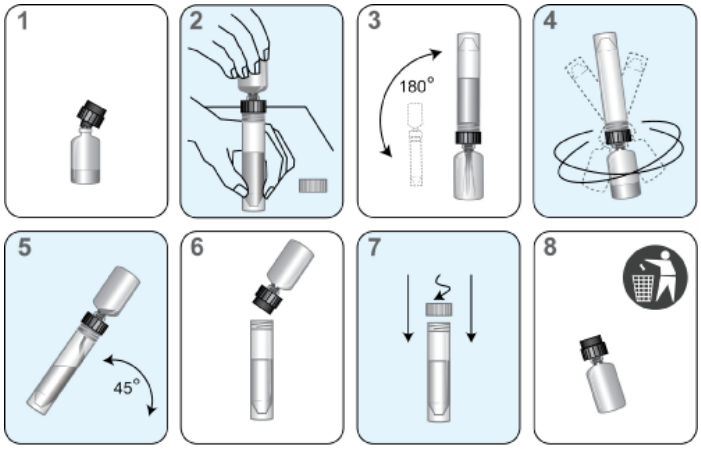

Figure 1. Panther System Reconstitution Process

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 in the kit 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 the Selection Reagent
   a. Check the reagent lot number on the Master Lot Barcode Sheet to make sure it belongs to the kit.
   b. If the 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 the 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 onto the system. Avoid creating foam during inversion of reagents.
6. Do not top off reagent bottles. The Panther System will recognize and reject bottles that have been topped off.

D. Sample Handling

1. Allow the samples (calibrators, specimens and any user provided external quality control samples) to reach room temperature prior to processing.
2. **Do not vortex samples**.
3. Inspect sample tubes before loading into the rack. 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 according to the instructions in the *Panther/Panther Fusion System Operator's Manual* and the *Procedural Notes* section below. Make sure that the appropriately sized reagent racks and TCR adapters are used.

Procedural Notes

A. Calibrators

1. To work properly with the Aptima HPV 16 18/45 genotype assay software on the Panther System, two replicates of the Negative Calibrator and each Positive Calibrator are required. One vial of each calibrator may be loaded in any rack position in a Sample Bay Lane on the Panther System. Specimen pipetting will begin when one of the following two conditions has been met:
   a. Positive and Negative Calibrators are currently being processed by the Panther System.
   b. Valid results for the calibrators are registered on the Panther System.
2. Once the calibrator tubes have been pipetted and are being processed for a specific reagent kit, specimens can be run with the associated assay reagent kit for up to 24 hours unless:
   a. Calibrators are invalid.
   b. The associated assay reagent kit is removed from the Panther System.
   c. The associated assay reagent kit has exceeded the stability limits.
3. Attempts to pipette more than two replicates from a calibrator tube can lead to insufficient volume errors.

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.

	Panther System
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	*≥ 800,000 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.

Analyte 16 Cutoff = *2 x [mean IC/16 RLU of the valid Negative Calibrator replicates] +*
0.1 x [mean IC/16 RLU of the valid Positive Calibrator 2 replicates]

E. 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.

Analyte 18/45 Cutoff = *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]

F. 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.

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

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.

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

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.

CSCT Kit specimens may be diluted to overcome potential inhibitory substances. Dilute 1 part of the invalid specimen into 8 parts of specimen transport media (the solution in CSCT Kit tubes); e.g. 560 µL of specimen into a new CSCT Kit tube which contains 4.5 mL of specimen transport media. Gently invert the diluted specimen to mix; avoid creating foam. Test the diluted specimen according to the standard assay procedure.

Note: *Do not dilute an invalid diluted specimen. If a diluted specimen yields an invalid result, a new specimen should be obtained from the patient.*

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. Specimen types other than those identified in the intended use have not been evaluated.

B. The performance of the Aptima HPV 16 18/45 genotype assay has not been evaluated for HPV vaccinated individuals.

C. The Aptima HPV 16 18/45 genotype assay has not been evaluated in cases of suspected sexual abuse.

D. 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.

E. 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.

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

G. The Internal Control monitors the target capture, amplification, and detection steps of the assay, It is not intended to control for cervical sampling adequacy.

H. 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.

I. 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.

J. 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.

K. 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.

L. 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.

M. High concentrations of HPV 45 can reduce the ability of the Aptima HPV 16 18/45 genotype assay to detect the presence of HPV 16 at low levels.

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

O. Use of this device may be limited to personnel trained in the use of the Aptima HPV 16 18/45 genotype assay.

P. Cross-contamination of samples can cause false positive results. The carryover rate of the Aptima HPV 16 18/45 genotype assay on the Panther System is 0.19%, as determined in a non-clinical study.

Q. The Aptima HPV 16 18/45 genotype assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

Panther System 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. 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 on the Panther System were evaluated at three testing sites with the Aptima HPV 16 18/45 genotype assay on the Panther System 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 study, based on results of testing with the Aptima HPV assay and the Aptima HPV 16 18/45 genotype assay on the Panther System, was categorized overall and by age group and by testing site. An Aptima HPV assay negative result on the Panther System indicates that none of the 14 high-risk HPV types are present, and were designated as Aptima HPV 16 18/45 genotype assay negative on the Panther System for the purpose of analysis. Results are shown in Table 1 for the ASC-US (atypical squamous cells of undetermined significance) and the NILM (negative for intraepithelial lesion or malignancy) 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 (71/911)	5.3 (48/911)	0.3 (3/911)	26.0 (237/911)	0.5 (50/10,839)	0.5 (49/10,839)	<0.1 (1/10,839)	3.6 (391/10,839)
Age Group (years)								
21 to 29	13.4 (52/388)	5.2 (20/388)	0.5 (2/388)	37.9 (147/388)	N/A	N/A	N/A	N/A
30 to 39	5.5 (14/255)	6.7 (17/255)	0.4 (1/255)	23.1 (59/255)	0.7 (31/4,183)	0.7 (31/4,183)	0 (0/4,183)	5.1 (215/4,183)
≥ 40	1.9 (5/268)	4.1 (11/268)	0 (0/268)	11.6 (31/268)	0.3 (19/6,656)	0.3 (18/6,656)	<0.1 (1/6,656)	2.6 (176/6,656)
Testing Site**								
1	5.6 (17/304)	6.6 (20/304)	0.3 (1/304)	27.0 (82/304)	0.4 (16/3,610)	0.4 (16/3,610)	<0.1 (1/3,610)	3.6 (130/3,610)
2	9.6 (29/303)	3.6 (11/303)	0.3 (1/303)	26.4 (80/303)	0.5 (18/3,614)	0.4 (15/3,614)	0 (0/3,614)	3.6 (130/3,614)
3	8.2 (25/304)	5.6 (17/304)	0.3 (1/304)	24.7 (75/304)	0.4 (16/3,615)	0.5 (18/3,615)	0 (0/3,615)	3.6 (131/3,615)

N/A = Not Applicable, HR = High-risk, Pos = Positive
Note: Women with Aptima HPV assay negative results on the Panther System were designated as Aptima HPV 16 18/45 genotype assay negative on the Panther System for purpose of analysis.
* HPV types 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and 68
** In the NILM population, not all subjects with Aptima HPV assay negative results on the Panther System were tested with the Aptima HPV 16 18/45 genotype assay on the Panther System. For the analysis by testing site, the results for these women were randomly assigned to one of the 3 testing sites.

Panther System Assay Performance

The Aptima HPV 16 18/45 genotype assay was first launched on the Tigris DTS System in 2012. In 2013, indications were expanded to use the Aptima HPV 16 18/45 genotype assay on the Panther System. The Panther System is an alternative, smaller instrument platform to the Tigris DTS System. Both systems are intended to fully automate amplified nucleic acid testing of diagnostic assays. Select assay performance testing completed on the Tigris DTS System was leveraged to support assay performance on the Panther System.

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

The Aptima HPV 16 18/45 genotype assay on the Panther System was evaluated using referral cytology specimens collected from consenting women during the prospective, multicenter US clinical study known as the CLEAR trial.

CLEAR Trial - Baseline Evaluation

The CLEAR trial was conducted to determine the clinical performance of the Aptima HPV assay on the Tigris DTS System for detection of cervical intraepithelial neoplasia grade 2 or more severe cervical disease (≥CIN2). The CLEAR trial included a baseline evaluation and a 3-year follow-up evaluation. 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 enrolled. At baseline, residual referral cytology specimens were tested with both the Aptima HPV assay on the Tigris DTS System and an FDA-approved HPV DNA test. These specimens were then divided into aliquots that were archived and stored at -70°C until they were tested with the Aptima HPV 16 18/45 genotype assay on the Panther System in the Aptima HPV 16 18/45 genotype assay clinical trial.

At baseline, all women in the ASC-US Study were referred to colposcopy, regardless of their Aptima HPV assay on the Tigris DTS System and FDA-approved 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 on the Tigris DTS System and/or the FDA-approved 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).

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. If the 3 pathologists disagreed, all 3 pathologists reviewed slides at a multi-headed microscope to reach consensus. Investigators, clinicians, and women were masked to the HPV test results until after completion of the colposcopy visit, to avoid bias.

At baseline, clinical performance of the Aptima HPV 16 18/45 genotype assay on the Panther System for detection of ≥CIN2 and cervical intraepithelial neoplasia grade 3 or more severe cervical disease (≥CIN3) was assessed relative to the cervical disease status determined at baseline.

CLEAR Trial – Follow-up Evaluation

Women in the NILM Study from 14 clinical sites were eligible to participate in the 3-year Follow-up Phase of the study if: i) they had a colposcopy visit at baseline and they did not have ≥CIN2, or ii) they did not have a colposcopy visit at baseline. The Follow-up Phase of the study consisted of annual visits. At these visits, cervical sampling was performed for each woman, and some women were also tested with an FDA-approved HPV test. Women with ASC-US or more severe cytology results during the follow-up period were referred to colposcopy using the same biopsy and histologic examination procedures performed for the baseline evaluation. Cervical disease status at a follow-up visit was considered “negative” based on NILM cytology or, for women with abnormal cytology test results, based on normal or CIN1 by consensus histology review panel. Women who had ≥CIN2 detected during the follow-up period were considered to have completed follow-up and did not attend visits after ≥CIN2 was detected. Women who did not have ≥CIN2 detected during the follow-up period but who attended a study visit in follow-up year 1 and/or follow-up year 2 and who attended a study visit in follow-up year 3 were considered to have completed follow-up.

The objective of the follow-up study was to compare the cumulative 3-year risk of cervical disease in women with baseline positive Aptima HPV assay and baseline positive Aptima HPV 16 18/45 genotype assay results with the cumulative 3-year risk of cervical disease in women with baseline positive Aptima HPV assay and baseline negative Aptima HPV 16 18/45 genotype assay results. The 3-year cervical disease status was determined as follows:

- Positive cervical disease status (≥CIN2 and/or ≥CIN3) – Women who had ≥CIN2 detected at baseline or during follow-up.
- Negative cervical disease status (<CIN2) – Women who completed follow-up without detection of ≥CIN2 and who were not considered to have “indeterminate” cervical disease status.
- Indeterminate cervical disease status – Women who had abnormal cytology test results during follow-up and who did not have a subsequent consensus histology review panel result, or women with inadequate cytology at their last visit.
- Lost to follow-up – Women who did not complete follow-up and who were not considered to have “indeterminate” cervical disease status.

Clinical performance of the Aptima HPV 16 18/45 genotype assay for detection of ≥CIN2 and ≥CIN3 was evaluated relative to the 3-year cervical disease status.

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

In total, there were 404 evaluable women 21 years of age and older with ASC-US cytology results and Aptima HPV assay positive results on the Panther System whose referral cytology samples were eligible for testing with the Aptima HPV 16 18/45 genotype assay on the Panther System. Of these, 45 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 353 evaluable women with conclusive disease status had valid Aptima HPV 16 18/45 genotype assay results on the Panther System based on reflex testing from an Aptima HPV assay positive result on the Panther System. Sixty-seven (67) women had ≥CIN2 and 30 had ≥CIN3.

Of the 353 evaluable women with Aptima HPV assay positive results on the Panther System, 118 women had Aptima HPV 16 18/45 genotype assay positive results on the Panther System indicating

the presence of HPV 16 and/or HPV 18/45; 235 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 539 evaluable women 21 years of age and older with ASC-US cytology results had Aptima HPV assay negative results on the Panther System. 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 on the Panther System for the purpose of analysis. Prevalence of ≥CIN2 and ≥CIN3 in evaluable women with ASC-US cytology results was 9.1% and 3.8% respectively. Based on testing with the Panther System, 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 Pos, HPV 18/45 Neg	HPV 16 Pos	1	26	18	11	15	0	71
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos	3	23	16	2	3	1	48
	HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	0	1	0	1	1	0	3
	HPV 16 Neg, HPV 18/45 Neg	Other HR HPV Pos	2	132	70	23	10	0	237
		Total	6	182	104	37	29	1	359
Negative	HPV 16/18/45 Neg***	HR HPV Neg	13	450	75	10	4	0	552
		Total	19	632	179	47	33	1****	911

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 is shown in Table 3. The risk of ≥CIN2 in women with HPV types 16, 18, and/or 45 present was 28.8% compared to 14.0% in women with one or more of the other 11 high-risk HPV types present and 2.6% in women with no high-risk HPV types present. Absolute risk is 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	28.8 (34/118) (22.2, 35.7)	16.9 (20/118) (12.1, 21.8)
	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	37.1 (26/70) (27.4, 47.4)	21.4 (15/70) (13.8, 29.5)
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	13.3 (6/45) (5.5, 25.1)	8.9 (4/45) (2.9, 19.1)
	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	14.0 (33/235) (10.7, 17.7)	4.3 (10/235) (2.3, 6.7)
	Pos or Neg	HR HPV Pos	19.0 (67/353) (16.8, 21.1)	8.5 (30/353) (7.1, 9.6)
Negative	HPV 16/18/45 Neg*	HR HPV Neg	2.6 (14/539) (1.5, 4.0)	0.7 (4/539) (0.2, 1.6)
		Prevalence	9.1% (81/892)	3.8% (34/892)

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**	27.4 (20/73) (19.0, 36.2)	16.4 (12/73) (10.3, 22.5)
		HPV 16 Pos, HPV 18/45 Neg	**HPV 16 Pos Only**	29.4 (15/51) (18.8, 41.1)	19.6 (10/51) (11.3, 28.5)
		HPV 16 Neg, HPV 18/45 Pos	**HPV 18/45 Pos Only**	15.0 (3/20) (3.6, 34.6)	5.0 (1/20) (0.2, 21.6)
		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**	17.1 (25/146) (12.7, 21.7)	5.5 (8/146) (2.8, 8.6)
		Pos or Neg	**HR HPV Pos**	20.5 (45/219) (17.9, 23.0)	9.1 (20/219) (7.5, 10.2)
	Negative	HPV 16/18/45 Neg*	**HR HPV Neg**	4.2 (7/166) (1.9, 7.6)	0.6 (1/166) (0.0, 2.7)
			Prevalence	13.5% (52/385)	5.5% (21/385)
30 to 39 Years	Positive	HPV 16 Pos and/or HPV 18/45 Pos	**HPV 16 and/or HPV 18/45 Pos**	30.0 (9/30) (16.5, 43.9)	16.7 (5/30) (6.9, 26.2)
		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**	13.3 (2/15) (1.3, 35.2)	13.3 (2/15) (1.3, 32.1)
		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**	12.1 (7/58) (5.7, 19.5)	3.4 (2/58) (0.5, 8.5)
		Pos or Neg	**HR HPV Pos**	18.2 (16/88) (13.4, 22.3)	8.0 (7/88) (4.6, 10.0)
	Negative	HPV 16/18/45 Neg*	**HR HPV Neg**	1.8 (3/163) (0.4, 4.3)	0.6 (1/163) (0.0, 2.4)
			Prevalence	7.6% (19/251)	3.2% (8/251)
≥ 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**	80.0 (4/5) (36.8, 99.0)	40.0 (2/5) (6.3, 78.2)
		HPV 16 Neg, HPV 18/45 Pos	**HPV 18/45 Pos Only**	10.0 (1/10) (0.4, 36.6)	10.0 (1/10) (0.4, 33.1)
		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**	3.2 (1/31) (0.1, 13.2)	0 (0/31) (0.0, 7.8)
		Pos or Neg	**HR HPV Pos**	13.0 (6/46) (6.1, 19.7)	6.5 (3/46) (1.7, 10.9)
	Negative	HPV 16/18/45 Neg*	**HR HPV Neg**	1.9 (4/210) (0.6, 3.4)	1.0 (2/210) (0.1, 2.0)
			Prevalence	3.9% (10/256)	2.0% (5/256)

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 is shown in Table 5. Women who had HPV types 16, 18, and/or 45 present were 11.1 times more likely to have ≥CIN2 and 22.8 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 2.1 times more likely to have ≥CIN2 and 4.0 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	11.1 (6.2, 20.0)	22.8 (8.0, 65.6)
HPV 16 and/or 18/45 Positive vs Other HR HPV Positive	2.1 (1.3, 3.1)	4.0 (1.9, 8.2)
Other HR HPV Positive vs HR HPV Negative	5.4 (2.9, 9.9)	5.7 (1.8, 18.1)
HR HPV Positive vs HR HPV Negative	7.3 (4.2, 12.8)	11.5 (4.1, 32.2)
Prevalence	9.1% (81/892)	3.8% (34/892)

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 4.1 times more likely to be present in a woman with ≥CIN2 and 5.2 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	4.1 (2.9, 5.6)	5.2 (3.5, 7.0)
Other HR HPV Positive	1.6 (1.2, 2.1)	1.1 (0.6, 1.8)
HR HPV Negative	0.3 (0.2, 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 at Baseline

In total, there were 512 evaluable women 30 years of age and older with NILM cytology results and Aptima HPV assay positive results on the Panther System whose referral cytology samples were eligible for testing with the Aptima HPV 16 18/45 genotype assay. Of these, 21 women (11 attended colposcopy and 10 did not attend colposcopy) did not have referral cytology sample volume available for testing in this study; after a missing values analysis, they were not included in the performance calculations. The 491 evaluable women had valid Aptima HPV 16 18/45 genotype assay results. Of these, 273 attended colposcopy. Fourteen (14) women had ≥CIN2 and 10 had ≥CIN3; 245 women had normal/CIN1 histology; 14 women had undetermined disease status.

Of the 259 evaluable women with conclusive disease status and Aptima HPV assay positive results on the Panther System at baseline, 65 had Aptima HPV 16 18/45 genotype assay positive results on the Panther System, indicating the presence of HPV 16 and/or HPV 18/45; 194 had negative results, indicating the presence of one or more of the other 11 high-risk HPV types. An additional 549 evaluable women 30 years of age and older with NILM cytology results and conclusive disease status had Aptima HPV assay negative results on the Panther System. 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 on the Panther System 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 at Baseline

Aptima HPV Assay Result	AHPV-GT Assay Result*	Interpretation	Consensus Histology Review Panel Diagnosis						
			Undetermined**	Normal	CIN1	CIN2	CIN3	Cancer	Total
Positive	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos	2	28	0	0	3	1	34
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos	1	28	1	1	0	2	33
	HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	0	1	0	0	0	0	1
	HPV 16 Neg, HPV 18/45 Neg	Other HR HPV Pos	11	175	12	3	4	0	205
		Total	14	232	13	4	7	3	273
Negative	HPV 16/18/45 Neg***	HR HPV Neg	31	527	16	5	1	0	580
		Total	45	759	29	9	8	3****	853

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).
**45 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=29), 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 491 women with Aptima HPV assay positive results on the Panther System and Aptima HPV 16 18/45 genotype assay results on the Panther System, 232 women had unverified (including undetermined) disease status (Table 8). Of the 10,348 women with Aptima HPV assay negative results from the original CLEAR trial, 9,799 had unverified disease status. Because the study was designed such that only randomly selected women with negative results for both the Aptima HPV assay on the Tigris DTS System and the FDA-approved DNA test were referred to colposcopy, the proportion of women with unverified disease status was high in this group (96.2%). 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 given the test results. For this method, missing disease status was imputed based on the results of the Aptima HPV assay on the Panther System, the Aptima HPV 16 18/45 genotype assay on the Panther System, and the FDA-approved HPV DNA test. Both verification-bias adjusted performance estimates and unadjusted performance estimates based on the 808 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 at Baseline

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	88	6	52	5	53	30 (34.1%)
	Positive	Negative	10	1	5	1	5	4 (40.0%)
	Positive	No Result**	2	0	1	0	1	1 (50.0%)
	Negative	Positive	291	7	169	4	172	115 (39.5%)
	Negative	Negative	85	0	14	0	14	71 (83.5%)
	Negative	No Result**	15	0	4	0	4	11 (73.3%)
		Total	491	14	245	10	249	232 (47.3%)
Negative	N/A***	Positive	282	3	177	1	179	102 (36.2%)
	N/A***	Negative	9,467	2	362	0	364	9,103 (96.2%)
	N/A***	No Result**	599	1	4	0	5	594 (99.2%)
		Total	10,839	20	788	11	797	10,031 (92.5%)

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).
**616 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) at baseline 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 9.7% compared to 3.2% in women with one or more of the other 11 high-risk HPV types present and 0.7% 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) at Baseline

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	9.7 (4.6, 20.2)	8.5 (3.8, 19.2)
	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	10.4 (4.0, 27.1)	10.3 (3.9, 27.1)
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	8.8 (2.9, 26.4)	6.5 (1.7, 25.1)
	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	3.2 (1.6, 6.3)	1.8 (0.6, 4.9)
	Pos or Neg	HR HPV Pos	4.6 (2.8, 7.4)	3.2 (1.7, 5.9)
Negative	HPV 16/18/45 Neg*	HR HPV Neg	0.7 (0.2, 2.5)	0.2 (0.0, 4.8)
		Prevalence	1.1%	0.8%

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) at Baseline

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	10.8 (7/65) (5.1, 17.7)	9.2 (6/65) (4.3, 14.2)
	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	12.5 (4/32) (3.7, 25.2)	12.5 (4/32) (3.9, 23.1)
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	9.4 (3/32) (2.2, 21.8)	6.3 (2/32) (0.9, 16.8)
	HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	0.0 (0/1) (0.0, 93.5)	0.0 (0/1) (0.0, 93.4)
	HPV 16/18/45 Neg	Other HR HPV Pos	3.6 (7/194) (1.7, 6.0)	2.1 (4/194) (0.7, 3.9)
	Pos or Neg	HR HPV Pos	5.4 (14/259) (3.7, 6.8)	3.9 (10/259) (2.6, 4.5)
Negative	HPV 16/18/45 Neg*	HR HPV Neg	1.1 (6/549) (0.5, 1.9)	0.2 (1/549) (0.0, 0.8)
		Prevalence	2.5% (20/808)	1.4% (11/808)

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) at Baseline

	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	8.1 (3/37) (2.0, 16.4)	5.4 (2/37) (0.9, 12.3)
		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	15.0 (3/20) (3.9, 30.6)	10.0 (2/20) (1.0, 22.8)
		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	3.6 (4/111) (1.2, 6.2)	2.7 (3/111) (0.7, 4.7)
		Pos or Neg	HR HPV Pos	4.7 (7/148) (2.6, 6.1)	3.4 (5/148) (1.6, 4.3)
	Negative	HPV 16/18/45 Neg*	HR HPV Neg	0.9 (2/230) (0.1, 2.2)	0.4 (1/230) (0.0, 1.6)
			Prevalence	2.4% (9/378)	1.6% (6/378)
≥ 40 Years	Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	14.3 (4/28) (4.8, 26.4)	14.3 (4/28) (5.0, 21.9)
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	26.7 (4/15) (6.4, 47.9)	26.7 (4/15) (6.5, 43.1)
		HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	0 (0/12) (0.0, 21.5)	0 (0/12) (0.0, 18.6)
		HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	0.0 (0/1) (0.0, 93.4)	0.0 (0/1) (0.0, 93.1)
		HPV 16/18/45 Neg	Other HR HPV Pos	3.6 (3/83) (1.0, 7.8)	1.2 (1/83) (0.0, 4.1)
		Pos or Neg	HR HPV Pos	6.3 (7/111) (3.3, 8.9)	4.5 (5/111) (2.3, 5.4)
	Negative	HPV 16/18/45 Neg*	HR HPV Neg	1.3 (4/319) (0.4, 2.3)	0 (0/319) (0.0, 0.8)
			Prevalence	2.6% (11/430)	1.2% (5/430)

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 HPV 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 12.9 times more likely to have ≥CIN2 and 53.3 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 3.0 times more likely to have ≥CIN2 and 4.8 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) at Baseline

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	12.9 (3.1, 54.6)	53.3 (1.5, >999)
HPV 16 and/or 18/45 Pos vs Other HR HPV Pos	3.0 (1.1, 8.8)	4.8 (1.2, 19.2)
Other HR HPV Pos vs HR HPV Neg	4.3 (1.2, 15.1)	11.0 (0.4, 289.2)
HR HPV Pos vs HR HPV Neg	6.1 (1.8, 21.0)	20.2 (0.7, 567.7)
Prevalence	1.1%	0.8%

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) at Baseline

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	9.9 (3.4, 28.4)	50.7 (6.2, 414.4)
HPV 16 and/or 18/45 Pos vs Other HR HPV Pos	3.0 (1.1, 8.2)	4.5 (1.3, 15.4)
Other HR HPV Pos vs HR HPV Neg	3.3 (1.1, 9.7)	11.3 (1.3, 100.7)
HR HPV Pos vs HR HPV Neg	4.9 (1.9, 12.7)	21.2 (2.7, 164.7)
Prevalence	2.5% (20/808)	1.4% (11/808)

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) at baseline by the Aptima HPV 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 11.2 times more likely to be present in a woman with ≥CIN2 and 24.1 times more likely to be present in a woman with ≥CIN3 at baseline.

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) at Baseline

Aptima Assay Result Interpretation*	≥CIN2	≥CIN3
	Likelihood Ratio (95% CI)	Likelihood Ratio (95% CI)
HPV 16 and/or 18/45 Positive	11.2 (3.3, 38.4)	24.1 (2.6, 225.9)
Other HR HPV Positive	3.5 (1.3, 9.4)	4.7 (0.7, 29.8)
HR HPV Negative	0.8 (0.6, 1.1)	0.4 (0.1, 2.2)

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) at Baseline

Aptima Assay Result Interpretation*	≥CIN2	≥CIN3
	Likelihood Ratio (95% CI)	Likelihood Ratio (95% CI)
HPV 16 and/or 18/45 Positive	4.8 (2.1, 8.5)	7.4 (3.3, 12.0)
Other HR HPV Positive	1.5 (0.7, 2.5)	1.5 (0.5, 2.9)
HR HPV Negative	0.4 (0.2, 0.8)	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.

NILM ≥ 30 Years Population: Aptima HPV 16 18/45 Genotype Assay Clinical Performance After 3 Years of Follow-up

There were 10,822 women 30 years of age and older with NILM cytology results and positive Aptima HPV assay results and valid Aptima HPV 16 18/45 genotype assay results or negative Aptima HPV assay results on the Panther System at baseline who were eligible for the Follow-up Phase. Of the women without ≥CIN2, 67.0% (7,235/10,802) of women completed a year 1 follow-up Pap visit, 60.3% (6,505/10,793) the year 2, and 58.7% (6,330/10,786) the year 3. Overall, 58.8% (6,366/10,822) women completed the study (had ≥CIN2 at baseline or during follow-up), and/or completed required visits.

Of the 10,822 subjects, 490 (4.5%) women had baseline Aptima HPV assay positive results and valid Aptima HPV 16 18/45 genotype assay results. Of these 490 women, 247 (50.4%) had either positive or negative 3-year disease status based on cytology or colposcopy/biopsy results. Twenty-five (25) women had ≥CIN2 including 18 with ≥CIN3; 222 women had normal/CIN1 histology.

Of the 247 evaluable women with 3-year disease status and positive Aptima HPV assay results, 47 (19.0%) had positive Aptima HPV 16 18/45 genotype assay results, indicating the presence of HPV 16 and/or HPV 18/45 above the clinical cutoff; 200 (81.0%) had negative results, indicating the presence of one or more of the other 11 high-risk HPV types above the clinical cutoff.

The remaining 10,332 women had negative Aptima HPV assay baseline results during the CLEAR trial. Of these, 57.6% (5,946/10,322) had a 3-year disease status. For the purpose of analysis, women with Aptima HPV assay negative results were designated as Aptima HPV 16 18/45 genotype assay negative. The results of the Aptima HPV 16 18/45 genotype assay at baseline and consensus histology review panel 3-year disease status (includes baseline and follow-up evaluation) are presented in Table 15.

Table 15: NILM ≥ 30 Years Population: Classification of Women Eligible for the Follow-up Phase by Baseline Results of the Aptima HPV 16 18/45 Genotype Assay and Aptima HPV Assay and Disease Status Determined in the Baseline and Follow-up Phases

Aptima HPV Assay Result	AHPV-GT Assay Result	Interpretation	3-year Disease Status (Includes Baseline and Follow-up Evaluation)							
			Lost to Follow-up	Indeterminate*	Normal	CIN1	CIN2	CIN3	Cancer	Total
Positive	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos	25	2	16	0	1	5	1	50
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos	22	3	18	2	2	0	2	49
	HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	1	0	0	0	0	0	0	1
	HPV 16 Neg, HPV 18/45 Neg	Other HR HPV Pos	168	22	178	8	4	10	0	390
		Total	216	27	212	10	7	15	3	490
Negative	HPV 16/18/45 Neg**	HR HPV Neg	4,150	236	5,879	46	16	5	0	10,332
		Total	4,366	263	6,091	56	23	20	3^	10,822

AHPV-GT = Aptima HPV 16 18/45 genotype assay; HR = High-risk; Neg = Negative; Pos = Positive
*Women who had abnormal cytology test results during follow up and who did not have a subsequent Consensus Histology Review Panel result, or women with inadequate cytology at their last visit
**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).

The 3-year cumulative risks of disease (≥CIN2 and ≥CIN3) are based on Kaplan-Meier estimation (life-table analysis) and include disease detected at baseline or in follow-up. Women who had some indication of disease (ASC-US or more severe cytology results) but with no consensus histology review

panel result were included in the analysis by using a multiple imputation method to predict the number of women with disease that would have been identified if the women had undergone colposcopy.

The 3-year cumulative absolute risks of disease (≥CIN2 and ≥CIN3) by Aptima HPV assay results and Aptima HPV 16 18/45 genotype assay result are shown in Table 16. The 3-year cumulative relative risk of disease for Aptima 16 18/45 genotype assay positive versus negative outcomes are shown in Table 17.

Table 16: NILM ≥ 30 Years Population: 3-year Cumulative Absolute Risk* of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV 16 18/45 Genotype Assay and Aptima HPV Assay at Baseline

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	16.5 (9.4, 28.1)	11.9 (6.0, 22.8)
	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	21.4 (10.8, 39.7)	18.6 (8.7, 37.3)
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	12.2 (4.7, 29.6)	5.4 (1.3, 21.1)
	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	5.7 (3.4, 9.5)	3.8 (2.0, 7.2)
	Pos or Neg	HR HPV Pos	7.9 (5.4, 11.3)	5.4 (3.5, 8.5)
Negative	HPV 16/18/45 Neg**	HR HPV Neg	0.3 (0.2, 0.5)	0.1 (0.0, 0.2)
		Prevalence	0.7%	0.3%

AHPV-GT = Aptima HPV 16 18/45 genotype assay; HR = High-risk; N/A = Not Applicable; Neg = Negative; Pos = Positive
*The 3-year cumulative risks adjusted for other possible biases were similar to the risks in this table. Because of anticipated differences in risks at year 1 and year 2 for the two groups of women in the follow-up study (those with colposcopy at baseline and those with no colposcopy at baseline), only the 3-year cumulative risk for the combined groups was reported.
**Women with Aptima HPV assay negative results were designated as Aptima HPV 16 18/45 genotype assay negative for the purpose of analysis.

Table 17: NILM ≥ 30 Years Population: 3-year Cumulative Relative Risk* of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV 16 18/45 Genotype Assay and Aptima HPV Assay at Baseline

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	51.2 (25.9, 101.0)	129.6 (42.7, 393.5)
HPV 16 and/or 18/45 Pos vs Other HR HPV Pos	2.9 (1.4, 6.2)	3.1 (1.2, 7.9)
Other HR HPV Pos vs HR HPV Neg	17.6 (8.9, 34.9)	42.0 (14.2, 124.0)
HR HPV Pos vs HR HPV Neg	24.3 (13.7, 43.2)	59.5 (22.0, 161.0)
Prevalence	0.7%	0.3%

CI = Confidence Interval; HR = High-risk; Neg = Negative; Pos = Positive
*The 3-year cumulative risks adjusted for other possible biases were similar to the risks in this table. Because of anticipated differences in risks at year 1 and year 2 for the two groups of women in the follow-up study (those with colposcopy at baseline and those with no colposcopy at baseline), only the 3-year cumulative risk for the combined groups was reported.
**Women with Aptima HPV assay negative results were designated as Aptima HPV 16 18/45 genotype assay negative for the purpose of analysis.

The 3-year cumulative prevalence of ≥CIN2 and ≥CIN3 in women with NILM cytology results at baseline were 0.7% and 0.3%, respectively. The relative risk of ≥CIN2 detection for women with HPV 16 and/or 18/45 positive results vs Other HR HPV Positive results was 2.9 (95% CI: 1.4, 6.2), indicating that ≥CIN2 was detected in women with HPV 16 and/or 18/45 positive results 2.9 times more frequently than in women with Other HR HPV positive results. The relative risk of ≥CIN3 was 3.1 (95% CI: 1.2, 7.9). The relative risk of ≥CIN2 detection for women with Other HR HPV positive results vs HR HPV Negative results was 17.6 (95% CI: 8.9, 34.9), indicating that ≥CIN2 was detected in women with Other HR HPV positive results 17.6 times more frequently than in women with HR HPV Negative results. The relative risk of ≥CIN3 was 42.0 (95% CI: 14.2, 124.0).

Aptima HPV 16 18/45 Genotype Assay Clinical Performance with SurePath Liquid Cytology Specimens

SurePath liquid cytology specimens were collected from Canadian women who were referred for follow-up due to one or more abnormal Pap tests, and HPV infection, or some other reason. An aliquot (0.5 mL) of each specimen was transferred into an Aptima Specimen Transfer tube and then treated using the Aptima Transfer Solution. A single replicate of each specimen was tested with the Aptima HPV assay (n=500). Positive samples were then tested with the Aptima HPV 16 18/45 genotype assay and Aptima HPV assay results are shown in Table 18. Similar results are shown for the commercially available HPV PCR test, which differentiates HPV 16 and HPV 18, but not HPV 45, separately from the other high-risk genotypes. The relative risk for disease for genotype positive versus negative outcomes are shown in Table 19 for the Aptima HPV 16 18/45 genotype assay and the HPV PCR test.

Table 18: Absolute Risk of ≥CIN3 for Results of the Aptima HPV 16 18/45 Genotype Assay and a Commercially Available HPV PCR Test

HR HPV Result	Genotype Result	Interpretation	Aptima Absolute Risk ≥CIN3 (95% CI)	HPV PCR Absolute Risk ≥CIN3 (95% CI)
Positive	HPV 16 Pos and/or HPV 18/45* Pos	HPV 16 and/or HPV 18/45* Pos	13.9 (10.8-17.0)	13.9 (11.4-16.4)
	HPV 16 Pos and HPV 18/45* Neg	HPV 16 Pos only	16.8 (12.4-21.3)	16.2 (12.8-19.5)
	HPV 16 Neg and/or HPV 18/45* Pos	HPV 18/45* Pos only	6.1 (2.0-12.9)	6.6 (2.1-13.9)
	HPV 16 Pos and/or HPV 18/45* Pos	HPV 16 and HPV 18/45* Pos	25.0 (2.9-59.8)	12.5 (1.3-34.5)
	HPV 16 Neg and/or HPV 18/45* Neg	Other HR HPV Pos	2.1 (1.4-2.8)	2.0 (1.4-2.7)
	Pos or Neg	HR HPV Pos	11.5 (10.3-12.4)	10.7 (9.8-11.4)
Negative**	HPV 16 Neg and/or HPV 18/45* Neg	HR HPV Neg	1.1 (0.5-2.0)	0.6 (0.2-1.4)
		Prevalence (%)	4.2%	4.6%

HR = high-risk; Pos = Positive; Neg = Negative
*HPV PCR test only differentiates HPV 16 and HPV 18 from the other 12 high-risk genotypes, including HPV 45.
**Women with Aptima HPV assay negative results were designated as Aptima HPV 16 18/45 genotype assay negative for the purpose of analysis.

Table 19: Relative Risk of ≥CIN3 for Results of the Aptima HPV 16 18/45 Genotype Assay and a Commercially Available HPV PCR Test

Aptima Assay Results		HPV PCR Test Results	
Test Interpretation	**Relative Risk ≥CIN3 (95% CI)**	**Test Interpretation**	**Relative Risk ≥CIN3 (95% CI)**
HPV 16 and/or 18/45 positive vs HR HPV negative	12.6 (5.9-27.0)	HPV 16 and/or 18/45 positive vs HR HPV negative	23.3 (8.4-64.3)
HPV 16 and/or 18/45 positive vs other HR HPV positive	3.0 (1.6-5.5)	HPV 16 and/or 18/45 positive vs other HR HPV positive	3.1 (1.8-5.3)
Other HR HPV positive vs HR HPV negative	4.2 (1.8-10.1)	Other HR HPV positive vs HR HPV negative	7.6 (2.6-22.4)
HR HPV positive vs HR HPV negative	8.3 (4.0-17.3)	HR HPV positive vs HR HPV negative	14.4 (5.3-39.5)
Prevalence	4.2%	Prevalence	4.6%

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

CSCT specimens were collected from women at routine screening or follow-up visits and tested with the Aptima HPV assay. Residual CSCT specimens (n=378) with a positive Aptima HPV assay result were tested with the Aptima HPV 16 18/45 Genoytpe Assay on the Tigris DTS System. The HPV genotype of each specimen was determined using a DNA genotyping test. Specimens with discordant results between the genotyping test (DNA and Aptima HPV 16 18/45 genotype assay) were tested with a validated reverse-transcriptase PCR sequencing test to resolve their HPV 16, HPV 18, and HPV 45 status. Clinical agreement (positive and negative) of the Aptima HPV 16 18/45 genotype assay for detection of high-risk HPV 16, 18 and 45 was determined. Results are presented in Table 20.

Table 20: 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 CSCT specimens

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

Pos = Positive, Neg = Negative
Positive agreement: 98.3% (176/179) (95% CI: 95.2, 99.4)
Negative agreement: 99.0% (197/199) (95% CI: 96.4, 99.7)

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 collected from women referred for a follow-up visit due to an abnormal Pap result. Specimens were initially tested with the Aptima HPV assay (n=651). Specimens with a positive Aptima HPV assay result (n=414) were then tested with the Aptima HPV 16 18/45 genotype assay on both the Tigris DTS System and the Panther System.

Clinical agreement of the Aptima HPV 16 18/45 genotype assay for detection of high-risk HPV 16, 18, and 45 for the Panther System was determined based on the Tigris DTS System result as the reference method. Positive and negative percent agreements and associated 95% Score confidence intervals were calculated. Results are presented in Table 21.

Table 21: Clinical agreement of the Aptima HPV 16 18/45 Genotype Assay on the Panther System for Detection of High-risk HPV 16, 18, and 45 in CSCT Specimens

		Tigris DTS System Result				
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Neg, HPV 18/45 Pos	HPV 16 Pos, HPV 18/45 Pos	HPV 16 Neg, HPV 18/45 Neg	Total
Panther System Result	HPV 16 Pos, HPV 18/45 Neg	194	0	1	3	198
	HPV 16 Neg, HPV 18/45 Pos	0	34	0	0	34
	HPV 16 Pos, HPV 18/45 Pos	0	0	7	0	7
	HPV 16 Neg, HPV 18/45 Neg	1	1	0	173	175
	Total	195	35	8	176	414

Pos = Positive, Neg = Negative
Positive agreement: 98.7% (235/238) (95% CI: 96.4, 99.6)
Negative agreement: 98.3% (173/176) (95% CI: 95.1, 99.4)

Comparison of the results from the Aptima HPV 16 18/45 Genotype Assay on the Panther System for Pre- and Post-cytology ThinPrep Clinical Samples

A study was conducted to assess the agreement of Aptima HPV 16 18/45 genotype assay results on the Panther System in cervical samples tested prior to (Pre-cytology) or after (Post-cytology) cytology processing on the ThinPrep 5000 Processor.

Samples were sourced from women who had cervical specimens collected and immersed in ThinPrep Pap Test vials as part of standard of care cervical cancer screening.

For each subject, two 1-mL aliquots of the cervical specimen stored in the ThinPrep Pap Test vial were manually transferred into an Aptima Specimen Transfer tube (Pre-cytology sample A and sample B). After processing with the ThinPrep 5000, one 1-mL of the residual ThinPrep specimen was transferred into an Aptima Specimen Transfer tube (Post-cytology sample C).

A total of 214 samples with positive Aptima HPV assay results were evaluated using the Aptima HPV 16 18/45 genotype assay. The frequency of HPV 16 and/or HPV 18/45 detected by the assay is shown in Table 22 for the total population, in Table 23 for the NILM (≥30 years) population, and in Table 24 for

the ASC-US (≥21 years) population. Only samples with positive Aptima HPV assay results for either sample A or sample B and positive for sample C were included in the analysis.

Table 22: Total Population[1]: Frequency of HPV 16 and/or 18/45 Genotypes Detected by the Aptima HPV 16 18/45 Genotype Assay in Pre- and Post-cytology Samples

		Pre-cytology Samples A and B			
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Neg, HPV 18/45 Pos	Other HR HPV[3] Pos, HPV 16/18/45 Neg	Undetermined[4]
Post-cytology Sample C[2]	HPV 16 Pos, HPV 18/45 Neg	18	0	0	2
	HPV 16 Neg, HPV 18/45 Pos	0	9	2	4
	HPV 16 Pos and HPV 18/45 Pos	0	0	0	1
	Other HR HPV[3] Pos, HPV 16/18/45 Neg	0	0	175	3

HR = high-risk, Neg = negative, Pos = positive.
[1] Total population includes >ASC-US, NILM, ASC-US.
[2] All samples have a complete set of results for a specimen on the Aptima HPV 16 18/45 genotype assay.
[3] HPV genotypes 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and/or 68.
[4] Includes samples where at least one pre-cytology sample (either A or B) is HPV 16 and/or HPV 18/45 negative.

Table 23: NILM ≥30 Years Population: Frequency of HPV 16 and/or 18/45 Genotypes Detected by the Aptima HPV 16 18/45 Genotype Assay in Pre- and Post-cytology Samples

		Pre-cytology Samples A and B			
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Neg, HPV 18/45 Pos	Other HR HPV[2] Pos, HPV 16/18/45 Neg	Undetermined[3]
Post-cytology Sample C[1]	HPV 16 Pos, HPV 18/45 Neg	5	0	0	2
	HPV 16 Neg, HPV 18/45 Pos	0	1	0	1
	Other HR HPV[2] Pos, HPV 16/18/45 Neg	0	0	71	2

HR = high-risk, Neg = negative, Pos = positive.
[1] All samples have a complete set of results for a specimen on the Aptima HPV 16 18/45 genotype assay.
[2] HPV genotypes 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and/or 68.
[3] Includes samples where at least one pre-cytology sample (either A or B) is HPV 16 and/or HPV 18/45 negative.

Table 24: ASC-US ≥21 Years Population: Frequency of HPV 16 and/or 18/45 Genotypes Detected by the Aptima HPV 16 18/45 Genotype Assay in Pre- and Post-cytology Samples

		Pre-cytology Samples A and B			
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Neg, HPV 18/45 Pos	Other HR HPV[2] Pos, HPV 16/18/45 Neg	Undetermined[3]
Post-cytology Sample C[1]	HPV 16 Pos, HPV 18/45 Neg	3	0	0	0
	HPV 16 Neg, HPV 18/45 Pos	0	3	1	1
	Other HR HPV[2] Pos, HPV 16/18/45 Neg	0	0	48	0

HR = high-risk, Neg = negative, Pos = positive.
[1] All samples have a complete set of results for a specimen on the Aptima HPV 16 18/45 genotype assay.
[2] HPV genotypes 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and/or 68.
[3] Includes samples where at least one pre-cytology sample (either A or B) is HPV 16 and/or HPV 18/45 negative.

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 or pools of negative clinical ThinPrep liquid cytology specimens spiked with HPV *in vitro* transcripts or HPV-infected cultured cells (SiHa, HeLa, and MS751; ATCC, Manassas, Virginia) at various concentrations. For *in vitro* transcript panels, 60 replicates of each copy level were tested with each of two reagent lots for a total of 120 replicates. For cell line panels, 30 replicates of each copy level were tested with each of two reagent lots for a total of 60 replicates. Testing was performed over eight days with a minimum of three runs performed each day and five replicates for a given genotype tested in each run. The 95% detection limit (Table 25) was calculated from Probit regression analysis of the positivity results for each dilutional panel.

Table 25: Limit of Detection at the Clinical Cutoff of the Aptima HPV 16 18/45 Genotype Assay

Target	Limit of Detection* (95% CI)
HPV 16	23.7 (19.1, 30.9)
HPV 18	26.1 (21.2, 33.9)
HPV 45	34.5 (28.5, 43.6)
SiHa	0.4 (0.3, 0.7)
HeLa	0.7 (0.4, 1.4)
MS751	0.2 (0.1, 0.3)

*copies per reaction for *in vitro* transcripts and cells per reaction for cell lines

Assay Precision

Aptima HPV 16 18/45 genotype assay precision was evaluated in two studies using the same 24-member panel. Study 1 was conducted at 3 external testing sites to determine assay reproducibility. Study 2 was conducted in-house to determine within laboratory precision. The panel included 17 HPV 16 and/or 18/45-positive members with concentrations at or above the limit of detection of the assay (expected positivity: ≥ 95%), 3 HPV 16 and/or 18/45-positive members with concentrations below the limit of detection of the assay (expected positivity: >0% to <25%), and 4 HPV-negative members. HPV 16 and/or 18/45-positive panel members were prepared by spiking *in vitro* transcript or 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 diluted with STM. HPV-negative panel members were prepared with pooled ThinPrep liquid cytology specimens or PreservCyt Solution diluted with STM.

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 2 reagent lots. 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 2 lots x 3 days x 3 replicates). In Study 2, testing was conducted in-house over 13 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 26a and Table 26b, along with a summary of the agreement with expected results for HPV 16 and HPV 18/45 respectively. Table 27 presents the HPV 16 and HPV 18/45 analyte S/CO values at the 2.5th, the 50th, and 97.5th percentiles of the S/CO distribution. The HPV 16 analyte S/CO variability is shown in Table 28 for Study 1 and Table 29 for Study 2 for the panel members with an expected positive result for HPV 16. The HPV 18/45 analyte S/CO variability is shown in Table 30 for Study 1 and Table 31 for Study 2 for the panel members with an expected positive result for HPV 18/45.

Table 26a: Aptima HPV 16 18/45 Genotype Assay Precision Study 1 and 2: Panel Description and Percent Agreement With HPV 16 Expected Results

Panel Description (copies or cells/reaction)	HPV 16 Expected Result	Percent Agreement (95% CI)	
		Study 1 (3 testing sites)	Study 2 (1 testing site)
HPV 16 IVT (240 copies) High-Positive	Positive	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV 18 IVT (260 copies) High-Positive	Negative	100 (107/107) (96.5, 100)	100 (162/162) (97.7, 100)
HPV 45 IVT (350 copies) High-Positive	Negative	99.1 (107/108) (94.9, 99.8)	99.4 (161/162) (96.6, 99.9)
HPV 16 clinical sample 1 High-Positive	Positive	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV 18/45 clinical sample 1 High-Positive	Negative	100 (108/108) (96.6, 100)	100 (161/161) (97.7, 100)
SiHa cells (4 cells) - High-Positive & HeLa cells (0.7 cells) - Low-Positive	Positive	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
SiHa cells (0.4 cells) - Low-Positive & HeLa cells (7 cells) - High-Positive	Positive	99.1 (107/108) (94.9, 99.8)	97.5 (158/162) (94.0, 99.1)
SiHa cells (0.4 cells) Low-Positive	Positive	99.1 (107/108) (94.9, 99.8)	97.5 (158/162) (94.0, 99.1)
HeLa cells (0.7 cells) Low-Positive	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
MS751 cells (0.2 cells) Low-Positive	Negative	100 (108/108) (96.6, 100)	99.4 (158/159) (96.5, 99.9)

Table 26a: Aptima HPV 16 18/45 Genotype Assay Precision Study 1 and 2: Panel Description and Percent Agreement With HPV 16 Expected Results *(continued)*

Panel Description (copies or cells/reaction)	HPV 16 Expected Result	Percent Agreement (95% CI)	
		Study 1 (3 testing sites)	Study 2 (1 testing site)
HPV 16 IVT (24 copies) Low-Positive	Positive	100 (107/107) (96.5, 100)	96.9 (157/162) (93.2, 98.7)
HPV 18 IVT (26 copies) Low-Positive	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV 45 IVT (35 copies) Low-Positive	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV 16 clinical sample 2 Low-Positive	Positive	98.1 (105/107) (93.4, 99.5)	98.8 (160/162) (95.7, 99.7)
HPV 16 clinical sample 3 Low-Positive	Positive	99.1 (107/108) (94.9, 99.8)	97.5 (158/162) (94.0, 99.1)
HPV 18/45 clinical sample 2 Low-Positive	Negative	100 (107/107) (96.5, 100)	100 (162/162) (97.7, 100)
HPV 18/45 clinical sample 3 Low-Positive	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
SiHa cells (0.001 cells) High-Negative	Negative	97.2 (105/108) (92.1, 99.1)	98.1 (158/161) (94.8, 99.4)
HeLa cells (0.001 cells) High-Negative	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
MS751 cells (0.006 cells) High-Negative	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV-negative clinical sample 1	Negative	100 (107/107) (96.5, 100)	100 (162/162) (97.7, 100)
HPV-negative clinical sample 2	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV-negative PreservCyt 1	Negative	100 (107/107) (96.5, 100)	100 (162/162) (97.7, 100)
HPV-negative PreservCyt 2	Negative	100 (107/107) (96.5, 100)	100 (162/162) (97.7, 100)

CI = Score Confidence Interval

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

Table 26b: Aptima HPV 16 18/45 Genotype Assay Precision Study 1 and 2: Panel Description and Percent Agreement With HPV 18/45 Expected Results

Panel Description (copies or cells/reaction)	Percent Agreement (95% CI)		
	HPV 18/45 Expected Result	Study 1 (3 testing sites)	Study 2 (1 testing site)
HPV 16 IVT (240 copies) High-Positive	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV 18 IVT (260 copies) High-Positive	Positive	100 (107/107) (96.5, 100)	100 (162/162) (97.7, 100)
HPV 45 IVT (350 copies) High-Positive	Positive	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV 16 clinical sample 1 High-Positive	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV 18/45 clinical sample 1 High-Positive	Positive	100 (108/108) (96.6, 100)	100 (161/161) (97.7, 100)
SiHa cells (4 cells) - High-Positive & HeLa cells (0.7 cells) - Low-Positive	Positive	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
SiHa cells (0.4 cells) - Low-Positive & HeLa cells (7 cells) - High-Positive	Positive	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
SiHa cells (0.4 cells) Low-Positive	Negative	100 (108/108) (96.6, 100)	99.4 (161/162) (96.6, 99.9)

Table 26b: Aptima HPV 16 18/45 Genotype Assay Precision Study 1 and 2: Panel Description and Percent Agreement With HPV 18/45 Expected Results *(continued)*

Panel Description (copies or cells/reaction)	Percent Agreement (95% CI)		
	HPV 18/45 Expected Result	Study 1 (3 testing sites)	Study 2 (1 testing site)
HeLa cells (0.7 cells) Low-Positive	Positive	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
MS751 cells (0.2 cells) Low-Positive	Positive	99.1 (107/108) (94.9, 99.8)	88.7 (141/159) (84.5, 93.5)
HPV 16 IVT (24 copies) Low-Positive	Negative	100 (107/107) (96.5, 100)	100 (162/162) (97.7, 100)
HPV 18 IVT (26 copies) Low-Positive	Positive	99.1 (107/108) (94.9, 99.8)	100 (162/162) (97.7, 100)
HPV 45 IVT (35 copies) Low-Positive	Positive	99.1 (107/108) (94.9, 99.8)	98.1 (159/162) (94.7, 99.4)
HPV 16 clinical sample 2 Low-Positive	Negative	100 (107/107) (96.5, 100)	100 (162/162) (97.7, 100)
HPV 16 clinical sample 3 Low-Positive	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV 18/45 clinical sample 2 Low-Positive	Positive	100 (107/107) (96.5, 100)	95.7 (155/162) (91.7, 98.0)
HPV 18/45 clinical sample 3 Low-Positive	Positive	100 (108/108) (96.6, 100)	98.8 (160/162) (95.6, 99.7)
SiHa cells (0.001 cells) High-Negative	Negative	100 (108/108) (96.6, 100)	100 (161/161) (97.7, 100)
HeLa cells (0.001 cells) High-Negative	Negative	97.2 (105/108) (92.1, 99.1)	98.1 (159/162) (94.7, 99.4)
MS751 cells (0.006 cells) High-Negative	Negative	75.0 (81/108) (66.1, 82.2)	88.3 (143/162) (84.2, 93.2)
HPV-negative clinical sample 1	Negative	99.1 (106/107) (94.9, 99.8)	100 (162/162) (97.7, 100)
HPV-negative clinical sample 2	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV-negative PreservCyt 1	Negative	100 (107/107) (96.5, 100)	100 (162/162) (97.7, 100)
HPV-negative PreservCyt 2	Negative	100 (107/107) (96.5, 100)	100 (162/162) (97.7, 100)

CI = Score Confidence Interval

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

Table 27: Aptima HPV 16 18/45 Genotype Assay Precision Study 1 and 2: Percentile Distribution of HPV 16 and HPV 18/45 Analyte S/CO Values

Panel Description (copies or cells/reaction)	HPV 16 Analyte S/CO Percentile						HPV 18/45 Analyte S/CO Percentile					
	Study 1 (3 testing sites)			Study 2 (1 testing site)			Study 1 (3 testing sites)			Study 2 (1 testing site)		
	2.5th	50th	97.5th	2.5th	50th	97.5th	2.5th	50th	97.5th	2.5th	50th	97.5th
HPV 16 IVT (240 copies) High-Positive	2.86	3.26	3.53	2.92	3.30	3.60	0.00	0.00	0.00	0.00	0.00	0.00
HPV 18 IVT (260 copies) High-Positive	0.00	0.30	0.59	0.13	0.34	0.51	5.22	5.66	8.86	5.24	5.53	6.17
HPV 45 IVT (350 copies) High-Positive	0.00	0.22	0.43	0.08	0.24	0.41	4.37	4.92	8.78	4.40	5.05	5.99
HPV 16 clinical sample 1 High-Positive	2.49	3.12	3.34	2.67	3.10	3.41	0.00	0.00	0.00	0.00	0.00	0.00
HPV 18/45 clinical sample 1 High-Positive	0.00	0.30	0.56	0.15	0.33	0.50	4.95	6.67	8.95	4.49	6.22	8.27
SiHa cells (4 cells) – High-Positive & HeLa cells (0.7 cells) – Low-Positive	2.48	3.26	3.60	2.83	3.29	3.62	3.76	4.64	6.16	4.12	4.58	5.28
SiHa cells (0.4 cells) – Low-Positive & HeLa cells (7 cells) – High-Positive	1.14	2.77	3.40	1.25	2.95	3.47	4.01	4.87	6.73	4.36	4.70	5.34
SiHa cells (0.4 cells) Low-Positive	1.60	2.81	3.24	1.13	2.70	3.26	0.00	0.00	0.09	0.00	0.00	0.00
HeLa cells (0.7 cells) Low-Positive	0.00	0.31	0.56	0.17	0.33	0.52	3.63	5.11	7.17	4.15	5.15	5.66
MS751 cells (0.2 cells) Low-Positive	0.00	0.26	0.41	0.12	0.28	0.38	1.33	4.23	6.28	0.34	3.34	5.38
HPV 16 IVT (24 copies) Low-Positive	1.56	3.16	3.43	0.99	3.16	3.57	0.00	0.00	0.00	0.00	0.00	0.00
HPV 18 IVT (26 copies) Low-Positive	0.00	0.30	0.52	0.14	0.30	0.51	4.76	5.48	8.01	4.47	5.42	5.86
HPV 45 IVT (35 copies) Low-Positive	0.00	0.24	0.43	0.12	0.24	0.39	1.57	4.81	8.91	2.04	4.80	5.85
HPV 16 clinical sample 2 Low-Positive	1.37	2.95	3.51	1.25	2.90	3.30	0.00	0.00	0.00	0.00	0.00	0.00
HPV 16 clinical sample 3 Low-Positive	1.80	2.96	3.58	1.15	2.84	3.26	0.00	0.00	0.00	0.00	0.00	0.00
HPV 18/45 clinical sample 2 Low-Positive	0.03	0.28	0.46	0.16	0.33	0.46	2.50	4.20	7.04	0.69	3.60	4.85
HPV 18/45 clinical sample 3 Low-Positive	0.00	0.32	0.54	0.14	0.32	0.48	2.37	4.83	8.07	1.68	4.08	7.21
SiHa cells (0.001 cells) High-Negative	0.28	0.32	1.12	0.28	0.31	0.43	0.00	0.00	0.04	0.00	0.00	0.02
HeLa cells (0.001 cells) High-Negative	0.28	0.33	0.43	0.29	0.32	0.36	0.00	0.00	1.28	0.00	0.00	0.87
MS751 cells (0.006 cells) High-Negative	0.17	0.32	0.35	0.27	0.32	0.36	0.00	0.01	4.32	0.00	0.01	2.03
HPV-negative clinical sample 1	0.24	0.32	0.35	0.28	0.31	0.35	0.00	0.00	0.03	0.00	0.00	0.02
HPV-negative clinical sample 2	0.27	0.32	0.35	0.29	0.32	0.34	0.00	0.00	0.03	0.00	0.00	0.03
HPV-negative PreservCyt 1	0.27	0.33	0.37	0.30	0.33	0.36	0.00	0.00	0.02	0.00	0.00	0.02
HPV-negative PreservCyt 2	0.29	0.33	0.37	0.30	0.33	0.35	0.00	0.00	0.02	0.00	0.00	0.01

Table 28: Aptima HPV 16 18/45 Genotype Assay Precision Study 1: HPV 16 Analyte Signal Variability for Panel Members with an Expected Positive Result for HPV 16

Panel Description (copies or cells/reaction)	N	Mean S/CO	Between Sites		Between Operators		Between Lots		Between Worklists		Within Worklists		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
HPV 16 IVT (240 copies) High-Positive	108	3.23	0.06	2.0	0.00	0.0	0.00	0.0	0.09	2.9	0.14	4.2	0.18	5.5
HPV 16 clinical sample 1 High-Positive	108	3.07	0.07	2.4	0.00	0.0	0.00	0.0	0.11	3.6	0.16	5.2	0.21	6.8
SiHa cells (4 cells) High-Positive & HeLa cells (0.7 cells) Low-Positive	108	3.22	0.10	3.2	0.02	0.6	0.00	0.0	0.08	2.4	0.21	6.5	0.25	7.6
SiHa cells (0.4 cells) Low-Positive & HeLa cells (7 cells) High-Positive	108	2.63	0.05	1.8	0.00	0.0	0.00	0.0	<0.01	0.0	0.58	22.3	0.59	22.3
SiHa cells (0.4 cells) Low-Positive	108	2.65	0.00	0.0	0.00	0.0	0.12	4.6	0.00	0.0	0.44	16.6	0.46	17.3
HPV 16 IVT (24 copies) Low-Positive	107*	3.01	0.06	2.1	0.05	1.5	0.05	1.6	0.00	0.0	0.44	14.6	0.45	14.9
HPV 16 clinical sample 2 Low-Positive	107*	2.88	0.08	2.8	0.00	0.0	0.08	2.9	0.17	5.9	0.39	13.7	0.44	15.4
HPV 16 clinical sample 3 Low-Positive	108	2.89	0.00	0.0	0.00	0.0	0.00	0.0	0.14	4.8	0.39	13.5	0.41	14.4

CV = Coefficient of Variation; SD = Standard Deviation

*Two samples had invalid Aptima HPV 16 18/45 genotype assay results and were not included in the analyses.

Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as zero.

Table 29: Aptima HPV 16 18/45 Genotype Assay Precision Study 2: HPV 16 Analyte Signal Variability for Panel Members with an Expected Positive Result for HPV 16

Panel Description (copies or cells/reaction)	N	Mean S/CO	Between Instruments		Between Operators		Between Lots		Between Worklists		Within Worklists		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
HPV 16 IVT (240 copies) High-Positive	162	3.28	0.05	1.5	0.02	0.5	0.12	3.8	0.17	5.3	0.13	3.8	0.25	7.7
HPV 16 clinical sample 1 High-Positive	162	3.08	0.04	1.2	0.00	0.0	0.08	2.6	0.07	2.3	0.19	6.2	0.22	7.2
SiHa cells (4 cells) High-Positive & HeLa cells (0.7 cells) Low-Positive	162	3.27	0.05	1.6	0.00	0.0	0.05	1.4	0.13	4.0	0.18	5.5	0.23	7.2
SiHa cells (0.4 cells) Low-Positive & HeLa cells (7 cells) High-Positive	162	2.78	0.08	2.8	0.04	1.3	0.28	10.2	0.20	7.1	0.53	18.9	0.64	22.8
SiHa cells (0.4 cells) Low-Positive	162	2.54	0.16	6.2	0.05	2.0	0.29	11.4	0.25	9.9	0.47	18.6	0.63	24.8
HPV 16 IVT (24 copies) Low-Positive	162	3.04	0.03	1.0	0.05	1.5	0.20	6.5	0.34	11.3	0.36	11.8	0.54	17.7
HPV 16 clinical sample 2 Low-Positive	162	2.77	0.08	2.9	0.00	0.0	0.23	8.3	0.21	7.5	0.37	13.3	0.49	17.7
HPV 16 clinical sample 3 Low-Positive	162	2.67	0.03	1.1	0.04	1.6	0.22	8.1	0.25	9.2	0.49	18.2	0.59	22.0

CV = Coefficient of Variation; SD = Standard Deviation

Note: *Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as zero.*

Table 30: Aptima HPV 16 18/45 Genotype Assay Precision Study 1: HPV 18/45 Analyte Signal Variability for Panel Members with an Expected Positive Result for HPV 18/45

Panel Description (copies or cells/reaction)	N	Mean S/CO	Between Sites		Between Operators		Between Lots		Between Worklists		Within Worklists		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
HPV 18 IVT (260 copies) High-Positive	107*	5.88	0.33	5.5	0.52	8.9	0.00	0.0	0.43	7.4	0.17	2.8	0.77	13.1
HPV 45 IVT (350 copies) High-Positive	108	5.12	0.43	8.4	0.47	9.2	0.31	6.1	0.58	11.3	0.18	3.6	0.93	18.2
HPV 18/45 clinical sample 1 High-Positive	108	6.71	0.66	9.8	0.58	8.7	0.50	7.5	0.42	6.2	0.94	14.0	1.44	21.5
SiHa cells (4 cells) High-Positive & HeLa cells (0.7 cells) Low-Positive	108	4.69	0.22	4.7	0.10	2.1	0.08	1.7	0.10	2.2	0.54	11.4	0.60	12.8
SiHa cells (0.4 cells) Low-Positive & HeLa cells (7 cells) High-Positive	108	4.94	0.28	5.7	0.00	0.0	0.00	0.0	0.38	7.7	0.42	8.4	0.63	12.7
HeLa cells (0.7 cells) Low-Positive	108	5.17	0.38	7.4	0.00	0.0	0.00	0.0	0.40	7.6	0.56	10.8	0.78	15.1
MS751 cells (0.2 cells) Low-Positive	108	4.00	0.62	15.4	0.00	0.0	0.38	9.5	0.47	11.8	0.94	23.5	1.28	31.9
HPV 18 IVT (26 copies) Low-Positive	108	5.52	0.21	3.8	0.15	2.7	0.00	0.0	0.37	6.7	0.60	10.9	0.75	13.7
HPV 45 IVT (35 copies) Low-Positive	108	4.71	0.34	7.1	0.41	8.6	0.15	3.1	0.69	14.6	0.88	18.6	1.24	26.3
HPV 18/45 clinical sample 2 Low-Positive	107*	4.29	0.17	4.0	0.00	0.0	0.00	0.0	0.38	8.9	1.05	24.6	1.13	26.5
HPV 18/45 clinical sample 3 Low-Positive	108	5.12	0.38	7.5	0.00	0.0	0.38	7.4	0.00	0.0	1.37	26.8	1.47	28.8

CV = Coefficient of Variation; SD = Standard Deviation

*Two samples had invalid Aptima HPV 16 18/45 genotype assay results and were not included in the analyses.

Note: *Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as zero.*

Table 31: Aptima HPV 16 18/45 Genotype Assay Precision Study 2: HPV 18/45 Analyte Signal Variability for Panel Members with an Expected Positive Result for HPV 18/45

Panel Description (copies or cells/reaction)	N	Mean S/CO	Between Instruments		Between Operators		Between Lots		Between Worklists		Within Worklists		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
HPV 18 IVT (260 copies) High-Positive	162	5.56	0.08	1.5	0.06	1.1	0.05	0.9	0.13	2.4	0.14	2.6	0.23	4.1
HPV 45 IVT (350 copies) High-Positive	162	5.09	0.16	3.1	0.00	0.0	0.54	10.6	0.46	9.1	0.12	2.3	0.74	14.5
HPV 18/45 clinical sample 1 High-Positive	161*	6.22	0.10	1.7	0.00	0.0	0.26	4.2	0.00	0.0	1.06	17.1	1.10	17.7
SiHa cells (4 cells) High-Positive & HeLa cells (0.7 cells) Low-Positive	162	4.59	0.00	0.0	0.07	1.5	0.07	1.4	0.20	4.3	0.23	5.0	0.32	6.9
SiHa cells (0.4 cells) Low-Positive & HeLa cells (7 cells) High-Positive	162	4.78	0.00	0.0	0.08	1.7	0.00	0.0	0.30	6.3	0.24	5.0	0.39	8.2
HeLa cells (0.7 cells) Low-Positive	162	5.08	0.08	1.5	0.00	0.0	0.00	0.0	0.15	3.0	0.31	6.1	0.35	7.0
MS751 cells (0.2 cells) Low-Positive	159*	3.19	0.18	5.7	0.36	11.2	0.71	22.4	0.15	4.7	1.36	42.6	1.59	50.0
HPV 18 IVT (26 copies) Low-Positive	162	5.38	0.00	0.0	0.00	0.0	0.00	0.0	0.23	4.4	0.25	4.7	0.35	6.4
HPV 45 IVT (35 copies) Low-Positive	162	4.79	0.31	6.4	0.11	2.3	0.55	11.4	0.62	13.0	0.50	10.5	1.02	21.4
HPV 18/45 clinical sample 2 Low-Positive	162	3.21	0.00	0.0	0.02	0.8	0.36	11.1	0.00	0.0	1.14	35.5	1.20	37.2
HPV 18/45 clinical sample 3 Low-Positive	162	4.09	0.00	0.0	0.00	0.0	0.00	0.0	0.15	3.6	1.33	32.6	1.34	32.8

CV = Coefficient of Variation; SD = Standard Deviation

*Two samples had invalid Aptima HPV 16 18/45 genotype assay results and were not included in the analyses.

Note: *Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as zero.*

Cross-Reactivity

Note: *Testing with potentially cross-reactive organisms for the Aptima HPV 16 18/45 genotype assay was performed using the Tigris DTS System. The Aptima HPV 16 18/45 genotype assay was first launched on the Tigris DTS System in 2012. In 2013, indications were expanded to use the Aptima HPV 16 18/45 genotype assay on the Panther System. The Panther System is an alternative, smaller instrument platform to the Tigris DTS System. Both systems are intended to fully automate amplified nucleic acid testing of diagnostic assays. Select assay performance testing completed on the Tigris DTS System was leveraged to support assay performance on the Panther System.*

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 Specimen 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 32. The study criteria for assessing the effect of the presence of microorganism on the specificity of the assay were based on positivity.

Table 32: 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 32: 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
Yeast/protozoa			
Candida albicans	$1x10^6$ CFU/mL	*Trichomonas vaginalis***	$1x10^5$ cells/mL
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		

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 32 which was also spiked with a low concentration of HPV infected SiHa cells (1.6 cell per 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

Note: *Testing with potential interfering substances for the Aptima HPV 16 18/45 genotype assay was performed using the Tigris DTS System. The Aptima HPV 16 18/45 genotype assay was first launched on the Tigris DTS System in 2012. In 2013, indications were expanded to use the Aptima HPV 16 18/45 genotype assay on the Panther System. The Panther System is an alternative, smaller instrument platform to the Tigris DTS System. Both systems are intended to fully automate amplified nucleic acid testing of diagnostic assays. Select assay performance testing completed on the Tigris DTS System was leveraged to support assay performance on the Panther System.*

The substances described in Table 33 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, intravaginal hormones (containing progesterone) at 1% w/v.

Table 33: 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 Specimen Transfer tube)
**Personal lubricant that contains 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 Systems Operator's Manual.

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Contact Information and Revision History

Hologic, Inc.
10210 Genetic Center Drive
San Diego, CA 92121 USA

Australian Sponsor Address:
Hologic (Australia & New Zealand) Pty Ltd
Macquarie Park NSW 2113

EC REP
Hologic BV
Da Vincilaan 5
1930 Zaventem
Belgium

For country-specific Technical Support and Customer Service email address and telephone number, visit www.hologic.com/support.

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

Serious incidents occurring in relation to the device in the European Union should be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

AW-22203-001 Rev. 001
2022-09

Revision History	Date	Description
AW-22203 Rev. 001	September 2022	• Created Aptima HPV-GT assay IFU AW-22203 Rev.001 based on AW-11504 Rev. 010 for regulatory compliance with IVDR. • Updated EU hazard information • Updated sections of General Information of Intended Use, Warnings and Precautions, Reagent Storage and Handling Requirements, Quality Control Procedure, Specimen Collection and Storage, Reagents and Materials Provided, Materials Required and Available Separately, and Panther System Assay Performance. • Updated Table 18 and Table 19 from Aptima HPV 16 18/45 Genotype Assay Clinical Performance with SurePath Liquid Cytology Specimens section. • Updated contact information including: EC Rep, CE Mark, Australian Rep information, and technical support.