Aptima HPV 16 18/45 Genotype Assay

For *in vitro* diagnostic use.

Rx only

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General Information – Tigris DTS System and Panther System

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) types 16, 18, and 45 in cervical specimens from women with Aptima HPV Assay positive results. The Aptima HPV 16 18/45 Genotype Assay can differentiate HPV 16 from HPV 18 and/or HPV 45, but does not differentiate between HPV 18 and HPV 45. Cervical specimens in ThinPrep® Pap Test vials containing PreservCyt® Solution and collected with broom-type or cytobrush/ spatula collection devices* may be tested with the Aptima HPV 16 18/45 Genotype Assay. The assay is used with the Tigris® DTS® System or the Panther® System.

The Aptima HPV 16 18/45 Genotype Assay is indicated for use for routine cervical cancer screening to assess the risk for cervical dysplasia and cancer, per professional medical guidelines, including triage of ASC-US cytology, co-testing (adjunctive screening) with cytology, and HPV primary screening of women to assess the risk for cervical pre-cancer and cancer.

Patients should be followed-up in accordance with professional medical guidelines, results from prior screening, medical history and other risk factors.

Primary HPV screening with the Aptima HPV 16 18/45 Genotype Assay has only been validated on the Panther System.

* Broom-type device (e.g., Wallach Papette®), or endocervical brush/spatula.

WARNING:
This test is not intended for use in determining the need for treatment (i.e. excisional or ablative treatment of the cervix) in the absence of high-grade cervical intraepithelial neoplasia (CIN). Women who are HPV 16/18/45 positive should be monitored carefully for the development of high-grade CIN according to current practice guidelines.

The Aptima HPV 16 18/45 Genotype Assay is not intended for use as a stand-alone assay. The assay should be performed only as a follow-up to an Aptima HPV Assay positive result.

The Aptima HPV 16 18/45 Genotype Assay is not intended for use in women under age 25 with normal cervical cytology.

The Aptima HPV 16 18/45 Genotype Assay is not intended to substitute for regular cervical cytology screening.

The use of this test has not been evaluated for the management of HPV vaccinated women, women with prior ablative or excisional therapy, hysterectomy, who are pregnant, or who have other risk factors (e.g. HIV+, immunocompromised, history of sexually transmitted infection).

Summary and Explanation of the Test

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

The HPV viral genome is a double-stranded circular DNA approximately 7900 base pairs in length. The genome has eight overlapping open reading frames. There are six early (E) genes, two late (L) genes, and one untranslated long control region. The L1 and L2 genes encode the major and minor capsid proteins. Early genes regulate HPV viral replication. The E6 and E7

genes of high-risk HPV genotypes are known oncogenes. Proteins expressed from E6/E7 polycistronic mRNA alter cellular p53 and retinoblastoma protein functions, leading to disruption of cell-cycle check points and cell genome instability.[5,6]

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

Studies have shown that different types of high-risk HPV confer different levels of risk for developing severe dysplasia or cervical carcinoma. World-wide, HPV types 16, 18, and 45 are associated with approximately 80% of all invasive cervical cancers. These three types are found in 75% of all squamous carcinomas, with type 16 alone found in over 60% of all squamous carcinomas. In adenocarcinomas, HPV types 16, 18, and 45 are found in 80-94% of cases, with types 18 and 45 comprising almost half of these infections.[2,10] The presence of HPV type 18 in early stage cervical cancer has been reported to be associated with a poor prognosis.[11] HPV types 18 and 45 are under-reported in precancerous lesions, which may be caused by the presence of occult lesions of the cervical canal inaccessible to colposcopic examination.[12] In women infected with HPV types 16 and/or 18, the cumulative risk of developing cervical disease is 10-fold higher compared to the risk for disease development due to other high-risk types.[13,14,15]

Principles of the Procedure

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

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

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

Detection of the amplicon is achieved by HPA using single-stranded nucleic acid probes with chemiluminescent labels that are complementary to the amplicon. The labeled nucleic acid

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

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

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. To reduce the risk of invalid results, carefully read the entire package insert and refer to the *Panther/Panther Fusion® System Operator's Manual for procedural information* prior to performing the assay on the Panther system.

C. Only personnel adequately trained in the use of the Aptima HPV Assay and in handling potentially infectious materials should perform this procedure. If a spill occurs, immediately disinfect following appropriate site procedures.

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

E. For additional specific warnings, precautions, and procedures to control for contamination for the Panther system, consult the *Panther/Panther Fusion System Operator's Manual.*

Laboratory Related

F. Use only supplied or specified disposable laboratory ware.

G. 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.

H. **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.

I. 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 *Tigris DTS System Test Procedure* or the *Panther System Test Procedure* for more information.

J. Use good standard practices for molecular laboratories including environmental monitoring.

Specimen Related

K. Test only the indicated specimen type. The Aptima HPV 16 18/45 Genotype Assay has only been validated for use with cervical specimens collected in PreservCyt Solution using a broom-type or cytobrush/spatula collection device.

L. 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. Aliquots subsequently removed from the ThinPrep Pap Test vial for testing with the Aptima HPV 16 18/45 Genotype Assay should be processed using only the Aptima Specimen Transfer Kit.

M. 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.

N. Expiration dates listed on specimen transfer kits and tubes pertain to the transfer site and not the testing facility. Specimens 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.

O. Adhere to Universal Precautions and established laboratory protocols for safe handling, disposal, and prevention of cross-contamination. Only personnel adequately trained in handling infectious materials should be permitted to handle specimens.

P. Change gloves immediately if they come into contact with a specimen. To prevent cross-contamination, discard used materials and avoid passing over any other containers.

Q. Upon piercing, liquid can discharge from tube caps upon piercing under certain conditions. Follow instructions in the Tigris DTS System Test Procedure or the Panther System Test Procedure to prevent this occurrence.

Assay Related

R. Use Universal Precautions when handling calibrators.

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

T. Avoid microbial and ribonuclease contamination of reagents.

U. Do not use kit after its expiration date.

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

W. Aptima Assay Fluids, Aptima Auto Detect Reagents, and Aptima System Fluid Preservative (Tigris DTS System only) are not part of the Master Lot; any lot may be used.

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

Y. Tips with hydrophobic plugs must be used.

Z. Some reagents of this kit are labeled with hazard information.

Note: *For information on any hazard and precautionary statements that may be associated with reagents refer to 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.*

US Hazard Information

Selection Reagent

Boric Acid 1 - 5%
Triton X-100 1 - 5%

DANGER
H315 – Causes skin irritation.
H360FD – May damage fertility. May damage the unborn child.
P264 – Wash face, hands and any exposed skin thoroughly after handling.
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of water and soap.
P321 – Specific treatment (see supplemental first aid instructions on this label).
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P362+ P364– Take off contaminated clothing and wash before reuse.
P201 – Obtain special instructions before use.
P202 – Do no handle until all safety precautions have been read and understood.
P308 + P313 – IF exposed or concerned: Get medical advice/attention.
P405 – Store locked up
P501 – Dispose of contents/container to an approved waste disposal plant.

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 table shows the storage conditions and stability for reagents and controls:

Reagent	Unopened Storage	Open Kit (Reconstituted)	
		Storage	Stability
Amplification Reagent	2ºC to 8ºC		
Enzyme Reagent	2ºC to 8ºC		
Probe Reagent	2ºC to 8ºC		
Internal Control Reagent	2ºC to 8ºC		
Positive and Negative Calibrators	2ºC to 8ºC		
Amplification Reconstitution Solution	15ºC to 30ºC	2ºC to 8ºC	30 days
Enzyme Reconstitution Solution	15ºC to 30ºC	2ºC to 8ºC	30 days
Probe Reconstitution Solution	15ºC to 30ºC	2ºC to 8ºC	30 days
Target Capture Reagent	15ºC to 30ºC	15ºC to 30ºC	30 days
Selection Reagent	15ºC to 30ºC		
Wash Solution	15ºC to 30ºC		
Oil Reagent	15ºC to 30ºC		
Buffer for Deactivation Fluid	15ºC to 30ºC		
Auto Detect Reagent 1	15ºC to 30ºC		
Auto Detect Reagent 2	15ºC to 30ºC		
Aptima System Fluid Preservative (Tigris DTS System Only)	15ºC to 30ºC		

B. After reconstitution, the Amplification Reagent, Enzyme Reagent and Probe Reagent are stable for 30 days when stored at 2°C to 8°C.

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

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

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

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

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

A. Specimen collection and processing

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. Aliquot removal for Aptima HPV 16 18/45 Genotype Assay.

	Panther System		Tigris DTS System	
Cytology Processor	**Pre-cytology Aliquot Removal**	**Post-cytology Aliquot Removal**	**Pre-cytology Aliquot Removal**	**Post-cytology Aliquot Removal**
ThinPrep 2000 System	Manual*	Manual*	Manual*	Manual*
ThinPrep 5000 Processor	Manual*	Manual*	Manual*	Manual*
ThinPrep 5000 Processor with AutoLoader	Manual*	Manual*	Manual*	Manual*
ThinPrep Genesis™ Processor	Manual* Or, Use ThinPrep Genesis Processor with "Aliquot" process or "Aliquot +Slide" process.	Manual* Or, Use ThinPrep Genesis Processor only with "Aliquot" process.	Manual* **Note:** The Aptima HPV16 18/45 Genotype Assay performance on pre-cytology ThinPrep liquid-based cytology specimen aliquot removed by ThinPrep Genesis Processor has not been evaluated with Tigris DTS System.	**Note:** The Aptima HPV 16 18/45 Genotype Assay performance on post-cytology (cytology by ThinPrep Genesis Processor) liquid-based cytology specimen aliquot removed by ThinPrep Genesis Processor has not been evaluated with Tigris DTS System.

*Manually transfer 1 mL of the ThinPrep liquid-based cytology specimen into an Aptima Specimen Transfer tube according to the instructions in the Aptima Specimen Transfer Kit package insert.

B. Transport and storage before testing

1. Transport the ThinPrep liquid cytology specimen 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 Aptima Specimen Transfer tube may be stored at -20°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.*

Tigris DTS System

Reagents and Materials Provided

Aptima HPV 16 18/45 Genotype Assay Kit, 100 tests Cat No. 303234 (3 boxes)

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

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

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

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

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

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

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

Materials Required But Available Separately

Materials available from Hologic have catalog numbers listed, unless otherwise specified.

Material	Cat. No.
Tigris DTS System	105118
Tigris DTS System Run Kit	301191
Multi-tube Units (MTU)	*104772-02*
MTU/Tiplet Waste Bag Kit	*900907*
MTU Waste Deflectors	*900931*
MTU Waste Cover	*105523*
Aptima Assay Fluids Kit *(Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)*	302382
Aptima Auto Detect Kit	301048
Aptima System Fluid Preservative Kit	302380
Tips, 1000 µL filtered, conductive, liquid sensing, and disposable	901121 (10612513 Tecan)
Not all products are available in all regions.	903031 (10612513 Tecan)
Contact your representative for region-specific information	MME-04128
	MME-04134 (30180117 Tecan)
Aptima Specimen Transfer Kit	301154C
Aptima Specimen Transfer Kit — printable	PRD-05110
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, minimum 5-8.25% (0.7 M to 1.16 M) sodium hypochlorite solution

Water for the Tigris DTS System
consult the Tigris DTS System Operator's Manual for specifications

Disposable gloves

Optional Materials

Material	Cat. No.
Hologic® Bleach Enhancer for Cleaning *For routine cleaning of surfaces and equipment*	302101
Tube Rocker	—
Plastic-backed bench covers	—

Tigris DTS System Test Procedure

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

A. Work Area Preparation

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

B. Reagent Reconstitution/Preparation of a New Kit

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

1. Prior to testing, Amplification, Enzyme, and Probe Reagents must be reconstituted by combining contents of the bottles of lyophilized reagent with the appropriate reconstitution solution.
   a. Allow the lyophilized reagents to reach room temperature (15°C to 30°C) before use.
   b. Pair each reconstitution solution with its lyophilized reagent. Ensure that the reconstitution solution and lyophilized reagent have matching label colors and symbols before attaching the reconstitution collar.
   c. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired. Label caps of reconstitution solution bottles.
   d. Open the lyophilized reagent glass vial and firmly insert the notched end of the reconstitution collar into the glass vial opening (Figure 1, Step 1).
   e. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.
   f. While holding the solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 1, Step 2).
   g. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 1, Step 3).
   h. Pick up the assembled bottles and swirl. Avoid creating foam while swirling the bottle (Figure 1, Step 4).
   i. Ensure the lyophilized reagent goes completely into solution. Swirl the bottles again and then slightly rock the solution within the glass vial back and forth to mix thoroughly.
   j. Visually check to see if reagent is completely in solution with no powder, clumps, or wavy lines.

k. Slowly tilt the assembled bottles again to allow all of the solution to drain back into the reconstitution solution bottle (Figure 1, Step 5).

l. Remove the reconstitution collar and glass vial (Figure 1, Step 6).

m. Recap the plastic bottle with either the saved, labeled cap that corresponds to the reagent or a new cap. Do not mismatch caps. Record operator initials and reconstitution date on the label (Figure 1, Step 7).

n. Discard the reconstitution collar and vial (Figure 1, Step 8).

o. Thoroughly mix each reagent by gently inverting prior to loading onto the Panther system.

Option: Additional mixing of the Amplification, Enzyme, and Probe reagents is allowed by placing the recapped plastic bottles on a tube rocker set at a moderate speed and tilt for a minimum of 5 minutes. Ensure reagents are thoroughly mixed.

WARNING: *Avoid creating foam when reconstituting reagents. Foam compromises the level-sensing in the Tigris DTS System. Adequate mixing of the reagents is necessary to achieve expected assay results.*

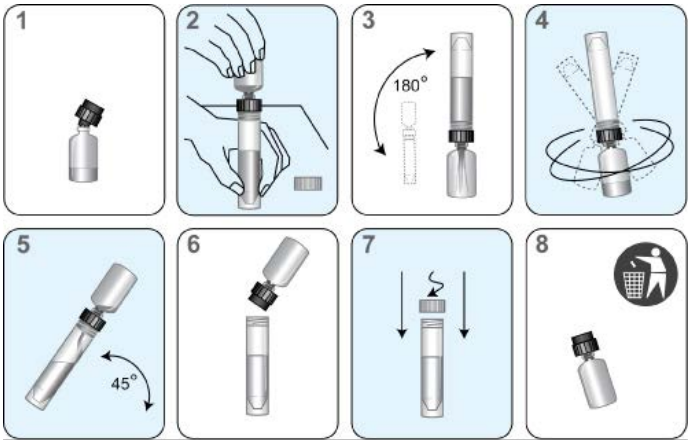

Figure 1. Tigris DTS System reconstitution process

2. Prepare the working Target Capture Reagent (wTCR):
   a. Pair the appropriate bottles of TCR and IC.
   b. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents are paired.
   c. Open the bottle of TCR, and set the cap on a clean, covered work surface.
   d. Open the bottle of IC and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the IC bottle.
   e. Cap the bottle of TCR and gently swirl the solution to mix the contents. Avoid creating foam during this step.
   f. Record operator initials and the current date on the label.
   g. Discard the IC bottle and cap.
   h. Precipitate may form in wTCR which may yield invalid results due to volume verification errors. Precipitate may be dissolved by warming wTCR at 42°C to 60°C for up to 90

minutes. Allow the wTCR to equilibrate to room temperature prior to use. Do not use if precipitate persists.

3. Prepare Selection Reagent
   a. Check the reagent lot number on the Master Lot Barcode Sheet to make sure it belongs to the kit.
   b. If 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.

Option: The reconstituted Amplification, Enzyme, and Probe reagents capped plastic bottles may be placed on a tube rocker set at a moderate speed and tilt for a minimum of 25 minutes to ensure reagents reach room temperature and are thoroughly mixed.

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 on the system. Avoid creating foam during inversion of reagents.
6. Do not top off reagent bottles. The Tigris DTS System will recognize and reject bottles that have been topped off.

WARNING: *Adequate mixing of the reagents is necessary to achieve expected assay results*.

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 racks. If a sample tube contains bubbles or has a lower volume than is typically observed, centrifuge the tube for 5 minutes at 420 RCF to ensure that no liquid is in the cap.

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

E. System Preparation

Set up the system and worklist according to the instructions in the Tigris DTS System Operator's Manual and the *Procedural Notes* section below. Make sure that the appropriately sized assay reagent wedge and TCR adapter are used.

Procedural Notes

A. Calibrators

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

B. Temperature

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

C. Glove Powder

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

Panther System

Reagents and Materials Provided

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

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

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

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

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

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

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

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

Materials Required But Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

Material	Cat. No.
Panther® System	303095
Panther Fusion® System	PRD-04172
Panther® System Continuous Fluids and Waste (Panther Plus)	PRD-06067
Panther System Run Kit	303096
Aptima Assay Fluids Kit	*303014*
(Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)	
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	901121 (10612513 Tecan)
Not all products are available in all regions.	903031 (10612513 Tecan)
Contact your representative for region-specific information	MME-04128
	MME-04134 (30180117 Tecan)
Aptima Specimen Transfer Kit	301154C
Aptima Specimen Transfer Kit — printable	PRD-05110
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, minimum 5-8.25% (0.7 M to 1.16 M) sodium hypochlorite solution	—
Disposable gloves	—

Optional Materials

Material	Cat. No.
Hologic® Bleach Enhancer for Cleaning *For routine cleaning of surfaces and equipment*	302101
Tube Rocker	—
Plastic-backed bench covers	—

Panther System Test Procedure

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

A. Work Area Preparation

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

B. Reagent Reconstitution/Preparation of a New Kit

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

1. Prior to testing, Amplification, Enzyme, and Probe Reagents must be reconstituted by combining contents of the bottles of lyophilized reagent with the appropriate reconstitution solution.
   a. Allow the lyophilized reagents to reach room temperature (15°C to 30°C) before use.
   b. Pair each reconstitution solution with its lyophilized reagent. Ensure that the reconstitution solution and lyophilized reagent have matching label colors and symbols before attaching the reconstitution collar.
   c. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired. Label caps of reconstitution solution bottles.
   d. Open the lyophilized reagent glass vial and firmly insert the notched end of the reconstitution collar into the glass vial opening (Figure 2, Step 1).
   e. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.
   f. While holding the solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 2, Step 2).
   g. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 2, Step 3).
   h. Pick up the assembled bottles and swirl. Avoid creating foam while swirling the bottle (Figure 2, Step 4).

i. Ensure the lyophilized reagent goes completely into solution. Swirl the bottles again and then slightly rock the solution within the glass vial back and forth to mix thoroughly.

j. Visually check to see if reagent is completely in solution with no powder, clumps, or wavy lines.

k. Slowly tilt the assembled bottles again to allow all of the solution to drain back into the reconstitution solution bottle (Figure 2, Step 5).

l. Remove the reconstitution collar and glass vial (Figure 2, Step 6).

m. Recap the plastic bottle with either the saved, labeled cap that corresponds to the reagent or a new cap. Do not mismatch caps. Record operator initials and reconstitution date on the label (Figure 2, Step 7).

n. Discard the reconstitution collar and glass vial (Figure 2, Step 8).

o. Thoroughly mix each reagent by gently inverting prior to loading onto the Panther system.

Option: Additional mixing of the Amplification, Enzyme, and Probe reagents is allowed by placing the recapped plastic bottles on a tube rocker set at a moderate speed and tilt for a minimum of 5 minutes. Ensure reagents are thoroughly mixed.

WARNING: *Avoid creating foam when reconstituting reagents. Foam compromises the level-sensing in the Panther System. Adequate mixing of the reagents is necessary to achieve expected assay results.*

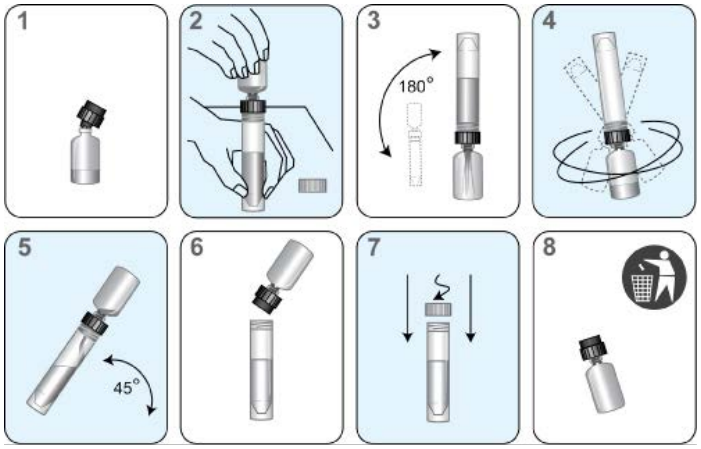

Figure 2. Panther System reconstitution process

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.

Option: The reconstituted Amplification, Enzyme, and Probe reagents capped plastic bottles may be placed on a tube rocker set at a moderate speed and tilt for a minimum of 25 minutes to ensure reagents reach room temperature and are thoroughly mixed.

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.

WARNING:*Adequate mixing of the reagents is necessary to achieve expected assay results.*

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/PantherFusion 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 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 – Tigris DTS System and Panther System

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.

Note: *Substantial reagent failure and system contamination may be indicated by invalid results for the Negative Calibrators, Positive Calibrators and/or the Internal Control. Follow instructions in Test Interpretation – Tigris DTS System and Panther System for retesting invalid results.*

Note: *External quality control samples (not provided) should be tested in conformance with local, state, and/or federal regulations or accreditation requirements and each laboratory's standard Quality Control procedures.*

External quality control samples can be prepared by spiking HPV infected cultured cells (i.e. SiHa, HeLa or MS751) into STM from an Aptima Specimen Transfer tube or into a matrix comprised of an HPV-negative ThinPrep liquid cytology specimen (or pool of specimens) diluted 1:2.9 with STM. Cells spiked at 25 cells/mL (10 cells per reaction) will monitor for substantial reagent failure, but will not necessarily monitor performance at the assay cutoff. Laboratories must establish acceptance criteria (e.g. percent positivity) for external quality control samples.

B. Calibrator Acceptance Criteria

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

	Tigris DTS System	Panther System
Negative Calibrator		
18/45 RLU	*≥ 0 and ≤ 60,000 RLU*	*≥ 0 and ≤ 60,000 RLU*
IC/16 RLU	*≥ 75,000 and ≤ 300,000 RLU*	*≥ 75,000 and ≤ 300,000 RLU*
Positive Calibrator 1		
18/45 RLU	*≥ 850,000 and ≤ 2,200,000 RLU*	*≥ 800,000 and ≤ 2,200,000 RLU*
IC/16 RLU	*≤ 475,000 RLU*	*≤ 475,000 RLU*
Positive Calibrator 2		
18/45 RLU	*≤ 115,000 RLU*	*≤ 115,000 RLU*
IC/16 RLU	*≥ 625,000 and ≤ 4,000,000 RLU*	*≥ 625,000 and ≤ 4,000,000 RLU*

RLU = Relative Light Units
IC = Internal Control

C. IC Cutoff

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

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

D. Analyte 16 Cutoff

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

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

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

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

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

F. Analyte 18/45 Cutoff

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

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

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

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

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

Test Interpretation – Tigris DTS System and Panther System

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 RLU and S/CO ratios as described in the table below. The first valid result is the result that should be reported. 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. If the result is invalid upon retest, a new specimen should be collected.

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*

*If post-cytology (post ThinPrep Genesis Processor cytology process) aliquot is used and patient has NILM cytology result, a false negative genotyping result may be obtained for samples close to the LoD (refer to "Samples Processed on the ThinPrep Genesis Processor".)

Note: *Results from user-provided external quality control samples must be monitored and assessed by laboratory personnel per laboratory procedures.*

Limitations – Tigris DTS System and Panther System

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

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

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

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

E. Aptima HPV 16 18/45 Genotype Assay performance has not been evaluated with post-processed ThinPrep liquid cytology specimens using processors other than the ThinPrep 2000 System, the ThinPrep 5000 Processor, the ThinPrep 5000 Processor with AutoLoader or the ThinPrep Genesis Processor.

F. Aptima HPV 16 18/45 Genotype Assay performance with Panther System has been evaluated for pre-cytology ThinPrep liquid-based cytology specimen aliquots removed by the ThinPrep Genesis Processor with "Aliquot+Slide" process or "Aliquot" process. Aptima HPV 16 18/45 Genotype Assay performance with Tigris DTS System has not been evaluated for pre-cytology ThinPrep liquid-based cytology specimen aliquots removed by the ThinPrep Genesis Processor.

G. Aptima HPV 16 18/45 Genotype Assay performance with Tigris DTS System and Panther System has been evaluated for post-cytology ThinPrep liquid-based cytology specimen aliquots manually removed after cytology processing by the ThinPrep 2000 System, the ThinPrep 5000 Processor, or the ThinPrep 5000 Processor with AutoLoader. Aptima HPV 16 18/45 Genotype Assay performance with Panther System has been evaluated for post-cytology (cytology by ThinPrep Genesis Processor) ThinPrep liquid-based cytology specimen aliquots removed manually or by "Aliquot" process on ThinPrep Genesis Processor. Aptima HPV 16 18/45 Genotype Assay performance with Tigris DTS System has not been evaluated for post-cytology (cytology by ThinPrep Genesis Processor) ThinPrep liquid-based cytology specimens.

H. When using the ThinPrep Genesis Processor, pre-processed aliquot samples are the preferred sample type, if available. There may be an increased risk of a false negative genotype result for HPV genotypes 16/18/45 in post-processed samples from patients with NILM cytology results.

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

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

K. 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.

L. The Aptima HPV 16 18/45 Genotype Assay provides qualitative results. Analyte levels are not necessarily associated with S/CO values (i.e., the expression level of mRNA in a specimen is not necessarily correlated with the magnitude of a positive assay signal). High S/CO values may be observed in samples close to the detection limit of the assay and low S/CO values may be observed in samples above the detection limit. Performing multiple tests on a sample may yield different S/CO values.

M. 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.

N. Infection with HPV is not an indicator of cytologic High-Grade Squamous Intraepithelial Lesion (HSIL) or underlying high-grade Cervical Intraepithelial Neoplasia (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.

O. 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.

P. 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.

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

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

S. Cross-contamination of samples can cause false positive results. The carryover rate of the Aptima HPV 16 18/45 Genotype Assay on the Tigris DTS System and the Panther System was 0.35% and 0.19% respectively, as determined in non-clinical studies.

T. The carry-over rate of specimens processed on the ThinPrep 2000 Processor prior to testing with the Aptima HPV 16 18/45 Genotype Assay on the Tigris DTS System was determined to be 0.6% when the cleaning procedure described in the Aptima Specimen Transfer Kit package insert was followed.

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

Tigris DTS 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.[19,20] Many studies have investigated HPV prevalence as determined by the detection of HPV DNA, however few studies report prevalence based on detection of HPV oncogenic mRNA. Women from a variety of clinical sites (n=18) representing a wide geographic distribution and a diverse population (10 states within the United States) were enrolled in a prospective clinical study known as the Clinical Evaluation of Aptima mRNA (CLEAR) trial to evaluate the Aptima HPV assay, which detects 14 high-risk HPV types.[21] Samples from women in the CLEAR trial with Aptima HPV assay positive results were evaluated at three testing sites with the Aptima HPV 16 18/45 Genotype Assay in a separate clinical study. The prevalence of HPV 16, 18/45, as well as the remaining 11 high-risk HPV types observed in the clinical study, based on results of testing with the Aptima HPV assay and the Aptima HPV 16 18/45 Genotype Assay, was categorized overall, by age group, and by testing site. Results are shown in Table 1 for the atypical squamous cells of undetermined significance (ASC-US) and the negative for intraepithelial lesion or malignancy (NILM) populations.

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

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

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

Aptima HPV 16 18/45 Genotype Assay on the Tigris DTS System Clinical Study Design

The Aptima HPV 16 18/45 Genotype Assay was evaluated using referral cytology specimens collected from consenting women during the prospective, multicenter US clinical study known as the CLEAR trial.[21]

CLEAR Trial – Baseline Evaluation

The CLEAR trial was conducted to determine the clinical performance of the Aptima HPV assay for detection of cervical intraepithelial neoplasia grade 2 or more severe cervical disease (≥CIN2). 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 from women in the ASC-US Study and in the NILM Study were tested with both the Aptima HPV assay 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 Tigris DTS 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 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 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 by 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 status. They were also masked to 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 Aptima HPV assay and FDA-approved HPV DNA 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 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 for cytology 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 Consensus Histology Review Panel results. 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.

Tigris DTS System Assay Performance

ASC-US ≥ 21 Years Population: Aptima HPV 16 18/45 Genotype Assay Clinical Performance

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

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

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

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

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

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

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

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

^One woman had adenocarcinoma in situ (AIS).

The absolute risk of disease (≥CIN2 and ≥CIN3) by Aptima HPV 16 18/45 Genotype Assay result and Aptima HPV assay result are shown in Table 3. The risk of ≥CIN2 in women with HPV types 16, 18, and/or 45 present was 29.1% compared to 14.3% in women with one or more of the other 11 high-risk HPV types present and 2.2% in women with no high-risk HPV types present. Absolute 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	29.1 (34/117) (22.4, 36.0)	16.2 (19/117) (11.4, 21.1)
	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	35.7 (25/70) (26.1, 45.9)	20.0 (14/70) (12.6, 28.0)
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	15.9 (7/44) (7.2, 28.3)	9.1 (4/44) (2.9, 19.5)
	HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	66.7 (2/3) (15.2, 98.2)	33.3 (1/3) (1.8, 84.6)
	HPV 16/18/45 Neg	Other HR HPV Pos	14.3 (33/231) (10.9, 17.9)	4.3 (10/231) (2.4, 6.8)
	Pos or Neg	HR HPV Pos	19.3 (67/348) (17.1, 21.3)	8.3 (29/348) (6.9, 9.4)
Negative	HPV 16/18/45 Neg*	HR HPV Neg	2.2 (12/545) (1.2, 3.5)	0.7 (4/545) (0.2, 1.6)
		Prevalence	8.8% (79/893)	3.7% (33/893)

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

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

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

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

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

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

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

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

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

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

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

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

NILM ≥ 30 Years Population: Aptima HPV 16 18/45 Genotype Assay Clinical Performance at Baseline

In total, there were 540 evaluable women 30 years of age and older with NILM cytology results and Aptima HPV assay positive results at baseline whose referral cytology samples were eligible for testing with the Aptima HPV 16 18/45 Genotype Assay. Of these, 25 women (18 attended colposcopy and 7 did not attend colposcopy) did not have referral cytology sample volume available for testing in this study; after a missing values analysis, they were not included in the performance calculations. The 515 evaluable women had valid Aptima HPV 16 18/45 Genotype Assay results. Of these, 317 attended colposcopy at baseline. Fifteen (15) women had ≥CIN2 and 10 had ≥CIN3; 283 women had normal/CIN1 histology; 19 women had undetermined disease status.

Of the 298 evaluable women with conclusive disease status and Aptima HPV assay positive results at baseline, 61 had Aptima HPV 16 18/45 Genotype Assay positive results, indicating the presence of HPV 16 and/or HPV 18/45; 237 had negative results, indicating the presence of one or more of the other 11 high-risk HPV types. An additional 505 evaluable women 30 years of age and older with NILM cytology results and conclusive disease status had Aptima HPV assay negative results at baseline during the CLEAR trial. An Aptima HPV assay negative result indicates that none of the 14 high-risk HPV types are present; women with Aptima HPV assay negative results were designated as Aptima HPV 16 18/45 Genotype Assay negative for the purpose of analysis. The results of the Aptima HPV 16 18/45 Genotype Assay by Aptima HPV assay result and Consensus Histology Review Panel diagnosis at baseline 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	27	0	0	3	1	33
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos	1	26	1	1	0	2	31
	HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	0	0	0	0	0	0	0
	HPV 16 Neg, HPV 18/45 Neg	Other HR HPV Pos	16	218	11	4	4	0	253
		Total	19	271	12	5	7	3	317
Negative	HPV 16/18/45 Neg***	HR HPV Neg	25	483	17	4	1	0	530
		Total	44	754	29	9	8	3^	847

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

Of the 515 women with Aptima HPV assay positive results and Aptima HPV 16 18/45 Genotype Assay results, 217 women had unverified (including undetermined) disease status at baseline (Table 8). Of the 10,331 women with Aptima HPV assay negative results from the original CLEAR trial, 9,826 had unverified disease status at baseline. Because the study was designed such that only randomly selected women with negative results for both the Aptima HPV assay and the FDA-approved DNA test were referred to colposcopy, the proportion of women with unverified disease status was high in this group (96.6%). To adjust for this verification bias, a multiple imputation method was used to predict the number of women with disease that would have been identified if all women had undergone colposcopy given the test results. Both verification-bias adjusted performance estimates and unadjusted performance estimates based on the 803 women with verified disease status at baseline 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	83	6	48	5	49	29 (34.9%)
	Positive	Negative	9	1	5	1	5	3 (33.3%)
	Positive	No Result**	2	0	1	0	1	1 (50.0%)
	Negative	Positive	271	7	171	4	174	93 (34.3%)
	Negative	Negative	137	1	52	0	53	84 (61.3%)
	Negative	No Result**	13	0	6	0	6	7 (53.8%)
		Total	515	15	283	10	288	217 (42.1%)
Negative	N/A***	Positive	306	3	178	1	180	125 (40.8%)
	N/A***	Negative	9,420	1	322	0	323	9,097 (96.6%)
	N/A***	No Result**	605	1	0	0	1	604 (99.8%)
		Total	10,846	20	783	11	792	10,043 (92.6%)

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

The adjusted absolute risks of disease (≥CIN2 and ≥CIN3) 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 12.6% compared to 3.4% in women with one or more of the other 11 high-risk HPV types present and 0.6% in women with no high-risk HPV types present. The unadjusted absolute risks of disease at baseline 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	12.6 (3.7, 21.4)	9.5 (2.1, 16.8)
	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	14.5 (2.1, 26.9)	12.1 (0.7, 23.4)
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	10.7 (0.0, 22.5)	6.9 (0.0, 16.2)
	HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	N/A	N/A
	HPV 16/18/45 Neg	Other HR HPV Pos	3.4 (1.2, 5.6)	1.8 (0.1, 3.5)
	Pos or Neg	HR HPV Pos	5.0 (2.6, 7.5)	3.2 (1.3, 5.2)
Negative	HPV 16/18/45 Neg*	HR HPV Neg	0.6 (0.1, 1.2)	0.4 (0.0, 0.7)
		Prevalence	0.9%	0.5%

AHPV-GT = Aptima HPV 16 18/45 Genotype Assay; HR = High-risk; N/A = Not Applicable; Neg = Negative; Pos = Positive
*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	11.5 (7/61) (5.4, 18.9)	9.8 (6/61) (4.6, 15.2)
	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	12.9 (4/31) (4.0, 26.0)	12.9 (4/31) (4.3, 23.8)
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	10.0 (3/30) (2.4, 23.0)	6.7 (2/30) (0.8, 17.7)
	HPV 16 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	3.4 (8/237) (1.7, 5.3)	1.7 (4/237) (0.6, 3.2)
	Pos or Neg	HR HPV Pos	5.0 (15/298) (3.6, 6.2)	3.4 (10/298) (2.3, 3.9)
Negative	HPV 16/18/45 Neg*	HR HPV Neg	1.0 (5/505) (0.4, 1.9)	0.2 (1/505) (0.0, 0.9)
		Prevalence	2.5% (20/803)	1.4% (11/803)

AHPV-GT = Aptima HPV 16 18/45 Genotype Assay; HR = High-risk; N/A = Not Applicable; Neg = Negative; Pos = Positive
*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	8.8 (3/34) (2.2, 17.8)	5.9 (2/34) (1.0, 13.3)
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	0 (0/17) (0.0, 15.5)	0 (0/17) (0.0, 14.3)
		HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	17.6 (3/17) (3.2, 35.4)	11.8 (2/17) (1.3, 27.0)
		HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	N/A (0/0)	N/A (0/0)
		HPV 16/18/45 Neg	Other HR HPV Pos	4.0 (5/124) (1.7, 6.2)	2.4 (3/124) (0.7, 4.2)
		Pos or Neg	HR HPV Pos	5.1 (8/158) (3.2, 6.1)	3.2 (5/158) (1.5, 4.0)
	Negative	HPV 16/18/45 Neg*	HR HPV Neg	0.5 (1/217) (0.0, 1.9)	0.5 (1/217) (0.0, 1.7)
			Prevalence	2.4% (9/375)	1.6% (6/375)
≥ 40 Years	Positive	HPV 16 Pos and/or HPV 18/45 Pos	HPV 16 and/or HPV 18/45 Pos	14.8 (4/27) (4.7, 27.3)	14.8 (4/27) (5.1, 22.8)
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	28.6 (4/14) (6.3, 50.7)	28.6 (4/14) (6.4, 46.5)
		HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	0 (0/13) (0.0, 20.1)	0 (0/13) (0.0, 17.1)
		HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	N/A (0/0)	N/A (0/0)
		HPV 16/18/45 Neg	Other HR HPV Pos	2.7 (3/113) (0.7, 5.8)	0.9 (1/113) (0.0, 3.1)
		Pos or Neg	HR HPV Pos	5.0 (7/140) (2.6, 7.0)	3.6 (5/140) (1.9, 4.2)
	Negative	HPV 16/18/45 Neg*	HR HPV Neg	1.4 (4/288) (0.5, 2.5)	0 (0/288) (0.0, 0.8)
			Prevalence	2.6% (11/428)	1.2% (5/428)

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

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

Table 11: NILM ≥ 30 Years Population: Relative Risk of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV 16 18/45 Genotype Assay and Aptima HPV Assay (Verification-Bias Adjusted Estimates) 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	20.9 (6.3, 69.3)	29.4 (7.2, 120.8)
HPV 16 and/or 18/45 Pos vs Other HR HPV Pos	3.7 (1.5, 9.5)	5.3 (1.5, 18.2)
Other HR HPV Pos vs HR HPV Neg	5.6 (1.8, 17.7)	5.6 (1.2, 26.0)
HR HPV Pos vs HR HPV Neg	8.5 (2.9, 24.8)	10.1 (2.7, 38.2)
Prevalence	0.9%	0.5%

CI = Confidence Interval; HR = High-risk; Neg = Negative; Pos = Positive
*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	11.6 (3.8, 35.4)	49.7 (6.1, 406)
HPV 16 and/or 18/45 Pos vs Other HR HPV Pos	3.4 (1.3, 9.0)	5.8 (1.7, 20.0)
Other HR HPV Pos vs HR HPV Neg	3.4 (1.1, 10.3)	8.5 (1.0, 75.8)
HR HPV Pos vs HR HPV Neg	5.1 (1.9, 13.8)	16.9 (2.2, 132)
Prevalence	2.5% (20/803)	1.4% (11/803)

CI = Confidence Interval; HR = High-risk; Neg = Negative; Pos = Positive
*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 16 18/45 Genotype Assay result are shown in Table 13 (verification-bias adjusted) and Table 14 (unadjusted). HPV types 16, 18, and/or 45 were 17.1 times more likely to be present in a woman with ≥CIN2 at baseline and 21.9 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	17.1 (6.2, 46.9)	21.9 (7.3, 65.2)
Other HR HPV Positive	4.2 (1.7, 10.1)	3.8 (1.2, 12.6)
HR HPV Negative	0.7 (0.5, 1.0)	0.7 (0.4, 1.1)

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

Table 14: NILM ≥ 30 Years Population: Likelihood Ratios and 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	5.1 (2.3, 9.1)	7.9 (3.5, 12.9)
Other HR HPV Positive	1.4 (0.7, 2.2)	1.2 (0.4, 2.3)
HR HPV Negative	0.4 (0.1, 0.7)	0.1 (0.0, 0.6)

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

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

There were 10,829 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 at baseline who were eligible for the Follow-up Phase. Of the women without ≥CIN2, 67.0% (7,237/10,809) of women completed a year 1 follow-up Pap visit, 60.3% (6,508/10,800) the year 2, and 58.7% (6,333/10,793) the year 3. Overall, 58.8% (6,369/10,829) of the women completed the study (had ≥CIN2 at baseline or during follow-up, and/or completed required visits).

Of the 10,829 evaluable subjects, 515 (4.8%) evaluable women had baseline Aptima HPV assay positive results and valid Aptima HPV 16 18/45 Genotype Assay results. Of these 515 women, 254 (49.3%) had either positive or negative 3-year disease status. Twenty-five (25) women had ≥CIN2 including 17 with ≥CIN3; 229 women had normal/CIN1 histology.

Of the 254 women with 3-year disease status and positive Aptima HPV assay baseline results, 43 (16.9%) 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; 211 (83.1%) had negative results, indicating the presence of one or more of the other 11 high-risk HPV types above the clinical cutoff.

The remaining 10,314 women had negative Aptima HPV assay baseline results during the CLEAR trial. Of these, 57.6% (5,943/10,314) had 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	23	2	15	0	1	5	1	47
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos	23	3	15	2	2	0	2	47
	HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	0	0	0	0	0	0	0	0
	HPV 16 Neg, HPV 18/45 Neg	Other HR HPV Pos	187	23	188	9	5	9	0	421
		Total	233	28	218	11	8	14	3	515
Negative	HPV 16/18/45 Neg**	HR HPV Neg	4,137	234	5,877	45	15	6	0	10,314
		Total	4,370	262	6,095	56	23	20	3^	10,829

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 detection (≥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 result 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	17.6 (10.1, 29.8)	12.7 (6.4, 24.3)
	HPV 16 Pos, HPV 18/45 Neg	HPV 16 Pos Only	22.5 (11.4, 41.4)	19.6 (9.2, 39.0)
	HPV 16 Neg, HPV 18/45 Pos	HPV 18/45 Pos Only	12.8 (4.9, 31.1)	5.7 (1.3, 22.2)
	HPV 16 Pos, HPV 18/45 Pos	HPV 16 & 18/45 Pos	N/A	N/A
	HPV 16/18/45 Neg	Other HR HPV Pos	5.2 (3.0, 8.7)	3.2 (1.6, 6.3)
	Pos or Neg	HR HPV Pos	7.4 (5.1, 10.7)	4.9 (3.1, 7.7)
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	53.7 (27.2, 106.2)	119.7 (41.1, 348.5)
HPV 16 and/or 18/45 Pos vs Other HR HPV Pos	3.4 (1.6, 7.3)	4.0 (1.5, 10.4)
Other HR HPV Pos vs HR HPV Neg	15.7 (7.9, 31.5)	30.3 (10.4, 88.2)
HR HPV Pos vs HR HPV Neg	22.6 (12.6, 40.4)	46.2 (17.7, 120.6)
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 3.4 (95% CI: 1.6, 7.3), indicating that ≥CIN2 was detected in women with HPV 16 and/or 18/45 positive results 3.4 times more frequently than in women with Other HR HPV positive results. The relative risk of ≥CIN3 was 4.0 (95% CI: 1.5, 10.4). The relative risk of ≥CIN2 detection for women with Other HR HPV positive results vs HR HPV Negative results was 15.7 (95% CI: 7.9, 31.5), indicating that ≥CIN2 was detected in women with Other HR HPV positive results 15.7 times more frequently than in women with HR HPV Negative results. The relative risk of ≥CIN3 was 30.3 (95% CI: 10.4, 88.2).

Agreement with Reverse Transcription-PCR Sequencing

The analytical performance of the Aptima HPV 16 18/45 Genotype Assay for detection of target was assessed against an in-house validated reverse transcription-polymerase chain reaction (RT-PCR) sequencing test specific for E6/E7 mRNA from the same 14 HR HPV types detected by the Aptima HPV assay. Sequencing was performed by an external commercial laboratory.

Cervical specimens collected at baseline from the ASC-US and NILM populations of the CLEAR trial from women with Aptima HPV assay positive results were tested with the RT-PCR sequencing test and compared to the Aptima HPV 16 18/45 Genotype Assay results. In total, 859 samples were tested: 354 from the ASC-US population and 505 from the NILM population.

For the ASC-US and NILM populations, Aptima HPV 16 18/45 Genotype Assay results by RT-PCR sequencing test results are shown in Table 18a and Table 18b, respectively. Positive and negative percent agreements for the ASC-US and NILM populations are shown in Table 18c and Table 18d, respectively.

Table 18a: ASC-US ≥ 21 Years Population: Comparison of Aptima HPV 16 18/45 Genotype Assay and RT-PCR Sequencing Test Results Including Only Samples With Aptima HPV Assay Positive Results

		RT-PCR Sequencing Test Results										
		One HR Type				Two HR Types				>2 HR Types		
Aptima HPV-GT Assay Result	No HR Type	16	18	45	Other HR	16 & Other	18 & Other	45 & Other	2 Other HR	≥1 of 16/18/45 Present	Only Other HR Present	Ind
16+, 18/45-	27	27	0	0	6	7	0	0	1	3	0	0
16-, 18/45+	4	0	17	9	4	0	4	4	1	4	0	0
16+, 18/45+	0	0	1	0	0	1	0	0	0	1	0	0
16-, 18/45-	76	0	1	1	128	0	2	1	16	0	6	2
Total	107	27	19	10	138	8	6	5	18	8	6	2

+ = Positive; - = Negative; HPV-GT = Aptima HPV 16 18/45 Genotype Assay; HR = high risk; Ind = indeterminate, unable to determine positivity for types 16/18/45 due to invalid RT-PCR sequencing test results.

Note: *Columns with all zeros are not shown*

Table 18b: NILM ≥ 30 Years Population: Comparison of Aptima HPV 16 18/45 Genotype Assay and RT-PCR Sequencing Test Results Including Only Samples With Aptima HPV Assay Positive Results

		RT-PCR Sequencing Test Results									
		One HR Type				Two HR Types				>2 HR Types	
Aptima HPV-GT Assay Result	No HR Type	16	18	45	Other HR	16 & Other	18 & Other	45 & Other	2 Other HR	≥1 of 16/18/45 Present	Ind
16+, 18/45-	24	19	0	0	2	1	0	0	0	0	0
16-, 18/45+	7	0	18	12	1	0	2	5	0	1	0
16+, 18/45+	0	0	0	0	0	0	0	0	0	0	0
16-, 18/45-	251	0	2	4	148	1	0	0	4	0	3
Total	282	19	20	16	151	2	2	5	4	1	3

+ = Positive; - = Negative; HPV-GT = Aptima HPV 16 18/45 Genotype Assay; HR = high risk; Ind = indeterminate, unable to determine positivity for types 16/18/45 due to invalid RT-PCR sequencing test results.

Note: *Columns with all zeros are not shown*

Table 18c: ASC-US ≥ 21 Years Population: Comparison of Aptima HPV 16 18/45 Genotype Assay and RT-PCR Sequencing Test Results Including Only Samples With Aptima HPV Assay Positive Results

RT-PCR Sequencing Test Results			
Aptima HPV-GT Assay Result	16/18/45-		
	16/18/45+	Other HR+	HR-
16/18/45+	78	12	31
16/18/45-	5	150	76
Total	83	162	107
Positive Percent Agreement: 94.0 (78/83) (95% CI: 86.7, 97.4)			
Negative Percent Agreement: 92.6 (150/162) (95% CI: 87.5, 95.7)			

+ = Positive; - = Negative; CI = Confidence Interval; HPV-GT = Aptima HPV 16 18/45 Genotype Assay; HR = high risk

Table 18d: NILM ≥ 30 Years Population: Comparison of Aptima HPV 16 18/45 Genotype Assay and RT-PCR Sequencing Test Results Including Only Samples With Aptima HPV Assay Positive Results

RT-PCR Sequencing Test Results			
Aptima HPV-GT Assay Result	16/18/45+	16/18/45- Other HR+	16/18/45- HR-
16/18/45+	58	3	31
16/18/45-	7	152	251
Total	65	155	282
Positive Percent Agreement: 89.2 (58/65) (95% CI: 79.4, 94.7)			
Negative Percent Agreement: 98.1 (152/155) (95% CI: 94.5, 99.3)			

+ = Positive; - = Negative; CI = Confidence Interval; HPV-GT = Aptima HPV 16 18/45 Genotype Assay; HR = high risk

Clinical Cutoff Determination for the Aptima HPV 16 18/45 Genotype Assay

The clinical cutoff for detecting high grade cervical disease (≥CIN2) for the Aptima HPV 16 18/45 Genotype Assay was verified with specimens from women with Aptima HPV assay positive results from the ASC-US and NILM populations in the CLEAR trial. The cutoff was set at 1.0 for both HPV 16 and HPV 18/45.

Limit of Detection at the Clinical Cutoff

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 determined by testing dilution panels of *in vitro* transcripts (IVT) for genotypes 16, 18 and 45 and 3 HPV-infected cell lines: SiHa, HeLa and MS751 (ATCC, Manassas, Virginia). For the IVT panels, specimen transport media was spiked with IVT at various concentrations and then diluted with individual negative ThinPrep liquid cytology specimens prior to testing. For the HPV-infected cell panels, pools of HPV-negative ThinPrep liquid cytology specimens were spiked with HPV-infected cells at various concentrations and then diluted with specimen transport media prior to testing. Thirty replicates of each copy level were tested with each of three reagent lots for a total of 90 replicates. Testing was performed over 6 days, with 3 runs performed per day and 5 replicates of a given genotype and concentration tested in each run. The 95% detection limit (Table 19) was calculated from Probit regression analysis of the positivity results for each dilutional panel.

Table 19: Limit of Detection at the Clinical Cutoff of the Aptima HPV 16 18/45 Genotype Assay

Target	Limit of Detection (95% CI)
HPV 16	57.3 copies/reaction (46.5, 74.6)
HPV 18	84.8 copies/reaction (66.1, 115.6)
HPV 45	60.0 copies/reaction (46.6, 82.3)
SiHa	1.15 cells/reaction (0.87, 1.69)
HeLa	0.38 cells/reaction (0.30, 0.53)
MS751	2.58 cells/reaction (1.87, 4.19)

CI = Confidence Interval

Assay Precision

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

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

The panel members are described in Table 20a and Table 20b, along with a summary of the agreement with expected results for HPV 16 and HPV 18/45 respectively. Table 21 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 22 for Study 1 and Table 23 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 24 for Study 1 and Table 25 for Study 2 for the panel members with an expected positive result for HPV 18/45.

Table 20a: Aptima HPV 16 18/45 Genotype Assay Precision Study 1 and 2: Panel Description and Percent Agreement With HPV 16 Expected Results

Panel Description (cells/reaction)	HPV 16 Expected Result	Percent Agreement (95% CI)	
		Study 1 (3 testing sites)	Study 2 (1 testing site)
SiHa cells (3.0 cells) Moderate-Positive	Positive	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HeLa cells (0.6 cells) Moderate-Positive	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
MS751 cells (11.0 cells) Moderate-Positive	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV 16 clinical sample 1 Moderate-Positive	Positive	100 (107/107) (96.5, 100)	100 (162/162) (97.7, 100)
HPV 18/45 clinical sample 1 Moderate-Positive	Negative	100 (108/108) (96.6, 100)	98.8 (160/162) (95.6, 99.7)
SiHa cells (1.6 cells) – Low-Positive & HeLa cells (3.3 cells) – High-Positive	Positive	100 (108/108) (96.6, 100)	98.8 (160/162) (95.6, 99.7)
SiHa cells (1.6 cells) – Low-Positive & MS751 cells (42.5 cells) – High-Positive	Positive	100 (108/108) (96.6, 100)	99.4 (161/162) (96.6, 99.9)
SiHa cells (15.7 cells) – High-Positive & HeLa cells (0.3 cells) – Low-Positive	Positive	100 (108/108) (96.6, 100)	100 (161/161) (97.7, 100)
SiHa cells (15.7 cells) – High-Positive & MS751 cells (4.3 cells) – Low-Positive	Positive	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
SiHa cells (1.6 cells) Low-Positive	Positive	97.2 (105/108) (92.1, 99.1)	98.8 (160/162) (95.6, 99.7)
HeLa cells (0.3 cells) Low-Positive	Negative	100 (108/108) (96.6, 100)	100 (161/161) (97.7, 100)
MS751 cells (4.3 cells) Low-Positive	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV 16 clinical sample 2 Low-Positive	Positive	97.2 (104/107) (92.1, 99.0)	94.4 (152/161) (88.7, 97.0)
HPV 18/45 clinical sample 2 Low-Positive	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
SiHa cells (0.1 cells) High-Negative	Negative	85.2 (92/108) (77.3, 90.7)	84.6 (137/162) (78.2, 89.3)
HeLa cells (0.02 cells) High-Negative	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
MS751 cells (0.04 cells) High-Negative	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV 16 clinical sample 3 High-Negative	Negative	95.4 (103/108) (89.6, 98.0)	92.6 (150/162) (87.5, 95.7)
HPV 18/45 clinical sample 3 High-Negative	Negative	100 (108/108) (96.6, 100)	99.4 (161/162) (96.6, 99.9)
HPV-negative clinical sample 1	Negative	100 (108/108) (96.6, 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 clinical sample 3	Negative	100 (108/108) (96.6, 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 20b: 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 (cells/reaction)	HPV 18/45 Expected Result	Percent Agreement (95% CI)	
		Study 1 (3 testing sites)	Study 2 (1 testing site)
SiHa cells (3.0 cells) Moderate-Positive	Negative	100 (108/108) (96.6, 100)	98.8 (160/162) (95.6, 99.7)
HeLa cells (0.6 cells) Moderate-Positive	Positive	93.5 (101/108) (87.2, 96.8)	98.1 (159/162) (94.7, 99.4)
MS751 cells (11.0 cells) Moderate-Positive	Positive	92.6 (100/108) (86.1, 96.2)	92.6 (150/162) (87.5, 95.7)
HPV 16 clinical sample 1 Moderate-Positive	Negative	100 (107/107) (96.5, 100)	100 (162/162) (97.7, 100)
HPV 18/45 clinical sample 1 Moderate-Positive	Positive	99.1 (107/108) (94.9, 99.8)	99.4 (161/162) (96.6, 99.9)
SiHa cells (1.6 cells) – Low-Positive & HeLa cells (3.3 cells) – High-Positive	Positive	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
SiHa cells (1.6 cells) – Low-Positive & MS751 cells (42.5 cells) – High-Positive	Positive	100 (108/108) (96.6, 100)	99.4 (161/162) (96.6, 99.9)
SiHa cells (15.7 cells) – High-Positive & HeLa cells (0.3 cells) – Low-Positive	Positive	63.9 (69/108) (54.5, 72.3)	67.7 (109/161) (60.1, 74.4)
SiHa cells (15.7 cells) – High-Positive & MS751 cells (4.3 cells) – Low-Positive	Positive	98.1 (106/108) (93.5, 99.5)	92.0 (149/162) (86.8, 95.3)
SiHa cells (1.6 cells) Low-Positive	Negative	100 (108/108) (96.6, 100)	99.4 (161/162) (96.6, 99.9)
HeLa cells (0.3 cells) Low-Positive	Positive	71.3 (77/108) (62.1, 79.0)	92.5 (149/161) (87.4, 95.7)
MS751 cells (4.3 cells) Low-Positive	Positive	86.1 (93/108) (78.3, 91.4)	69.1 (112/162) (61.6, 75.7)
HPV 16 clinical sample 2 Low-Positive	Negative	100 (107/107) (96.5, 100)	99.4 (160/161) (96.6, 99.9)
HPV 18/45 clinical sample 2 Low-Positive	Positive	88.0 (95/108) (80.5, 92.8)	79.6 (129/162) (72.8, 85.1)
SiHa cells (0.1 cells) High-Negative	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HeLa cells (0.02 cells) High-Negative	Negative	92.6 (100/108) (86.1, 96.2)	86.4 (140/162) (80.3, 90.9)
MS751 cells (0.04 cells) High-Negative	Negative	97.2 (105/108) (92.1, 99.1)	98.1 (159/162) (94.7, 99.4)
HPV 16 clinical sample 3 High-Negative	Negative	100 (108/108) (96.6, 100)	99.4 (161/162) (96.6, 99.9)
HPV 18/45 clinical sample 3 High-Negative	Negative	80.6 (87/108) (72.1, 86.9)	81.5 (132/162) (74.8, 86.7)
HPV-negative clinical sample 1	Negative	100 (108/108) (96.6, 100)	99.4 (161/162) (96.6, 99.9)
HPV-negative clinical sample 2	Negative	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HPV-negative clinical sample 3	Negative	100 (108/108) (96.6, 100)	99.4(161/162) (96.6, 99.9)

CI = Score Confidence Interval

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

Table 21: 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 (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
SiHa cells (3.0 cells) Moderate-Positive	1.43	3.30	3.89	2.21	3.36	3.86	0.00	0.00	0.25	0.00	0.00	0.00
HeLa cells (0.6 cells) Moderate-Positive	0.02	0.26	0.49	0.02	0.27	0.46	0.37	3.96	5.33	1.09	3.95	5.17
MS751 cells (11.0 cells) Moderate-Positive	0.25	0.37	0.64	0.22	0.36	0.57	0.68	3.67	4.51	0.61	2.80	4.29
HPV 16 clinical sample 1 Moderate-Positive	2.70	3.74	4.17	3.53	3.94	4.42	0.00	0.00	0.00	0.00	0.00	0.00
HPV 18/45 clinical sample 1 Moderate-Positive	0.05	0.36	0.71	0.08	0.35	0.71	1.24	4.68	7.25	1.62	4.08	6.20
SiHa cells (1.6 cells) – Low-Positive & HeLa cells (3.3 cells) – High-Positive	1.44	3.43	4.34	1.28	3.22	4.35	3.24	4.20	5.01	3.07	4.04	5.04
SiHa cells (1.6 cells) – Low-Positive & MS751 cells (42.5 cells) – High-Positive	1.53	3.28	4.14	1.41	3.26	4.18	3.14	3.78	4.37	2.77	3.69	4.23
SiHa cells (15.7 cells) – High-Positive & HeLa cells (0.3 cells) – Low-Positive	3.11	3.81	4.47	3.35	4.01	4.75	0.00	1.86	4.08	0.00	1.75	4.11
SiHa cells (15.7 cells) – High-Positive & MS751 cells (4.3 cells) – Low-Positive	3.02	3.90	4.54	3.42	4.01	4.64	1.18	3.27	4.34	0.64	2.89	3.95
SiHa cells (1.6 cells) Low-Positive	0.83	3.30	3.91	1.23	3.23	3.90	0.00	0.00	0.01	0.00	0.00	0.13
HeLa cells (0.3 cells) Low-Positive	0.04	0.30	0.49	0.00	0.26	0.47	0.00	2.63	4.81	0.44	3.57	4.95
MS751 cells (4.3 cells) Low-Positive	0.16	0.35	0.59	0.23	0.34	0.51	0.25	2.34	4.48	0.17	1.69	3.75
HPV 16 clinical sample 2 Low-Positive	0.89	2.78	3.63	0.82	2.66	3.95	0.00	0.00	0.00	0.00	0.00	0.00
HPV 18/45 clinical sample 2 Low-Positive	0.24	0.34	0.63	0.23	0.34	0.56	0.44	2.58	4.41	0.27	2.37	4.43
SiHa cells (0.1 cells) High-Negative	0.28	0.31	2.70	0.27	0.33	2.62	0.00	0.00	0.07	0.00	0.00	0.07
HeLa cells (0.02 cells) High-Negative	0.25	0.31	0.38	0.18	0.30	0.35	0.00	0.02	2.72	0.00	0.01	2.42
MS751 cells (0.04 cells) High-Negative	0.25	0.31	0.38	0.27	0.31	0.35	0.00	0.00	1.03	0.00	0.00	0.84
HPV 16 clinical sample 3 High-Negative	0.25	0.31	3.38	0.28	0.32	3.07	0.00	0.00	0.60	0.00	0.00	0.11
HPV 18/45 clinical sample 3 High-Negative	0.26	0.31	0.53	0.19	0.33	0.80	0.00	0.08	4.39	0.00	0.11	4.70
HPV-negative clinical sample 1	0.27	0.31	0.35	0.28	0.31	0.34	0.00	0.00	0.15	0.00	0.00	0.16
HPV-negative clinical sample 2	0.27	0.31	0.35	0.28	0.31	0.34	0.00	0.00	0.07	0.00	0.00	0.09
HPV-negative clinical sample 3	0.26	0.30	0.34	0.27	0.30	0.33	0.00	0.00	0.05	0.00	0.00	0.30

Table 22: 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 (cells/reaction)	N	Mean S/CO	Between Sites		Between Operators		Between Worklists		Within Worklists		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
SiHa cells (3.0 cells) Moderate-Positive	108	3.19	0.00	0.0	0.21	6.7	0.24	7.6	0.42	13.1	0.53	16.6
HPV 16 clinical sample 1 Moderate-Positive	107*	3.72	0.07	1.8	0.05	1.4	0.17	4.5	0.21	5.7	0.28	7.6
SiHa cells (1.6 cells) Low-Positive & HeLa cells (3.3 cells) High-Positive	108	3.23	0.00	0.0	0.16	4.8	0.24	7.4	0.70	21.7	0.76	23.4
SiHa cells (1.6 cells) Low-Positive & MS751 cells (42.5 cells) High-Positive	108	3.14	0.14	4.6	0.19	6.2	0.30	9.6	0.56	17.9	0.68	21.7
SiHa cells (15.7 cells) High-Positive & HeLa cells (0.3 cells) Low-Positive	108	3.79	0.10	2.7	0.00	0.0	0.22	5.8	0.26	7.0	0.36	9.4
SiHa cells (15.7 cells) High-Positive & MS751 cells (4.3 cells) Low-Positive	108	3.88	0.11	2.9	<0.01	0.2	0.04	1.0	0.33	8.4	0.35	9.0
SiHa cells (1.6 cells) Low-Positive	108	2.93	0.20	6.7	0.29	9.9	0.28	9.7	0.76	26.1	0.89	30.3
HPV 16 clinical sample 2 Low-Positive	107*	2.58	0.24	9.5	0.08	3.2	0.24	9.4	0.77	29.8	0.85	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.*

Table 23: 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 (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 (%)
SiHa cells (3.0 cells) Moderate-Positive	162	3.27	0.00	0.0	0.00	0.0	0.00	0.0	0.16	4.8	0.43	13.1	0.46	13.9
HPV 16 clinical sample 1 Moderate-Positive	162	3.95	0.06	1.6	0.09	2.2	0.15	3.8	0.09	2.2	0.24	6.0	0.31	7.9
SiHa cells (1.6 cells) Low-Positive & HeLa cells (3.3 cells) High-Positive	162	3.08	0.17	5.5	0.15	4.8	0.28	9.0	0.49	16.0	0.59	19.2	0.85	27.5
SiHa cells (1.6 cells) Low-Positive & MS751 cells (42.5 cells) High-Positive	162	3.08	0.00	0.0	0.00	0.0	0.15	4.9	0.50	16.2	0.59	19.0	0.78	25.4
SiHa cells (15.7 cells) High-Positive & HeLa cells (0.3 cells) Low-Positive	161*	4.02	0.15	3.7	0.08	2.1	0.18	4.5	0.07	1.8	0.30	7.6	0.40	9.9
SiHa cells (15.7 cells) High-Positive & MS751 cells (4.3 cells) Low-Positive	162	4.01	0.10	2.5	0.05	1.2	0.13	3.3	0.00	0.0	0.31	7.7	0.35	8.8
SiHa cells (1.6 cells) Low-Positive	162	2.98	0.09	3.0	0.13	4.2	0.30	10.2	0.37	12.3	0.57	19.1	0.76	25.4
HPV 16 clinical sample 2 Low-Positive	161*	2.58	0.00	0.0	0.00	0.0	0.29	11.1	0.54	20.9	0.67	25.9	0.91	35.1

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 24: 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 (cells/reaction)	N	Mean S/CO	Between Sites		Between Operators		Between Worklists		Within Worklists		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
HeLa cells (0.6 cells) Moderate-Positive	108	3.62	0.00	0.0	0.36	9.9	0.00	0.0	1.30	35.9	1.35	37.2
MS751 cells (11.0 cells) Moderate-Positive	108	3.30	0.00	0.0	0.39	11.9	0.00	0.0	1.03	31.1	1.10	33.3
HPV 18/45 clinical sample 1 Moderate-Positive	108	4.61	0.00	0.0	0.28	6.1	0.00	0.0	1.35	29.3	1.38	29.9
SiHa cells (1.6 cells) Low-Positive & HeLa cells (3.3 cells) High-Positive	108	4.19	0.04	1.1	0.15	3.6	0.00	0.0	0.41	9.9	0.44	10.6
SiHa cells (1.6 cells) Low-Positive & MS751 cells (42.5 cells) High-Positive	108	3.80	0.08	2.0	0.09	2.4	0.14	3.8	0.29	7.8	0.35	9.2
SiHa cells (15.7 cells) High-Positive & HeLa cells (0.3 cells) Low-Positive	108	1.86	0.00	0.0	0.46	24.8	0.00	0.0	1.32	71.0	1.40	75.3
SiHa cells (15.7 cells) High-Positive & MS751 cells (4.3 cells) Low-Positive	108	3.07	0.00	0.0	<0.01	0.0	0.26	8.4	0.76	24.9	0.81	26.3
HeLa cells (0.3 cells) Low-Positive	108	2.40	0.00	0.0	0.45	18.6	0.00	0.0	1.61	67.2	1.67	69.8
MS751 cells (4.3 cells) Low-Positive	108	2.39	0.00	0.0	0.30	12.6	0.41	17.1	1.10	45.9	1.21	50.6
HPV 18/45 clinical sample 2 Low-Positive	108	2.61	0.00	0.0	0.23	9.0	0.16	5.9	1.19	45.5	1.22	46.7

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 25: 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 (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 (%)
HeLa cells (0.6 cells) Moderate-Positive	162	3.67	0.15	3.9	0.05	1.3	0.46	12.6	0.67	18.3	0.74	20.1	1.11	30.2
MS751 cells (11.0 cells) Moderate-Positive	162	2.69	0.05	1.8	0.00	0.0	0.00	0.0	0.34	12.8	1.02	38.0	1.08	40.1
HPV 18/45 clinical sample 1 Moderate-Positive	162	4.01	0.17	4.2	0.00	0.0	0.20	4.9	0.74	18.4	0.97	24.1	1.24	31.0
SiHa cells (1.6 cells) Low-Positive & HeLa cells (3.3 cells) High-Positive	162	4.06	0.26	6.4	0.07	1.7	0.21	5.0	0.14	3.3	0.43	10.6	0.56	13.9
SiHa cells (1.6 cells) Low-Positive & MS751 cells (42.5 cells) High-Positive	162	3.63	0.20	5.5	0.00	0.0	0.09	2.5	0.10	2.9	0.38	10.4	0.45	12.3
SiHa cells (15.7 cells) High-Positive & HeLa cells (0.3 cells) Low-Positive	161*	1.71	0.00	0.0	0.28	16.1	0.34	19.6	0.85	49.9	0.79	46.4	1.25	72.7
SiHa cells (15.7 cells) High-Positive & MS751 cells (4.3 cells) Low-Positive	162	2.62	0.31	11.9	0.00	0.0	0.17	6.6	0.24	9.1	0.89	33.8	0.98	37.6
HeLa cells (0.3 cells) Low-Positive	161*	3.25	0.31	9.5	0.17	5.3	0.31	9.4	0.75	23.1	0.89	27.2	1.25	38.5
MS751 cells (4.3 cells) Low-Positive	162	1.84	0.00	0.0	0.00	0.0	0.21	11.5	0.44	24.1	1.02	55.4	1.13	61.5
HPV 18/45 clinical sample 2 Low-Positive	162	2.38	0.44	18.6	0.00	0.0	0.00	0.0	0.95	39.8	0.90	37.8	1.38	58.0

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.*

A third study was also conducted to determine within laboratory precision by testing a 9-member panel comprised of in vitro transcript (IVT) spiked into a matrix of PreservCyt solution diluted 1:2.9 in STM. One panel member was HPV negative. Four panel members were low positive, with IVT (HPV 16, HPV 18, HPV 45, HPV 18 and HPV 45) spiked at the limit of detection of the assay (expected positivity: ≥ 95%). Four panel members were moderate positive, with IVT (HPV 16, HPV 18, HPV 45, HPV 18 and HPV 45) spiked above the limit of detection of the assay (~3 x the detection limit; expected positivity: 100%). Testing was conducted in-house by 3 operators using 2 reagent lots, 3 instruments, over 9 days, testing 2 runs per day in which the panel was tested in triplicate. The panel members are described in Table 26, along with a summary of the agreement with expected results (HPV 16 and HPV 18/45). The S/CO values at the 2.5th, 50th, and 97.5th percentiles of the signal distribution are shown in Table 27. The HPV 16 analyte S/CO variability is shown in Table 28 for the panel members with an expected positive result for HPV 16. The HPV 18/45 analyte S/CO variability is shown in Table 29 for the panel members with an expected positive result for HPV 18/45.

Table 26: Aptima HPV 16 18/45 Genotype Assay Precision Study 3: Agreement with Expected Results

Description (copies/reaction)	Expected Result	Valid N	HPV 16 Agreement			HPV 18/45 Agreement		
			N	%	95% CI	N	%	95% CI
HPV 16 IVT (60 copies) Low-Positive	16 Pos, 18/45 Neg	108	108	100	96.6, 100	108	100	96.6, 100
HPV 18 IVT (85 copies) Low-Positive	16 Neg, 18/45 Pos	108	108	100	96.6, 100	108	100	96.6, 100
HPV 45 IVT (60 copies) Low-Positive	16 Neg, 18/45 Pos	108	108	100	96.6, 100	108	100	96.6, 100
HPV 18 IVT (85 copies) Low-Positive & HPV 45 IVT (60 copies) Low-Positive	16 Neg, 18/45 Pos	108	107	99.1	94.9, 99.8	108	100	96.6, 100
HPV Negative (0 copies)	16 Neg, 18/45 Neg	108	108	100	96.6, 100	108	100	96.6, 100
HPV 16 IVT (180 copies) Moderate-Positive	16 Pos, 18.45 Neg	108	108	100	96.6, 100	108	100	96.6, 100
HPV 18 IVT (260 copies) Moderate-Positive	16 Neg, 18/45 Pos	108	108	100	96.6, 100	108	100	96.6, 100
HPV 45 IVT (180 copies) Moderate-Positive	16 Neg, 18/45 Pos	108	108	100	96.6, 100	108	100	96.6, 100
HPV 18 IVT (260 copies) Moderate-Positive & HPV 45 IVT (180 copies) Moderate-Positive	16 Neg, 18/45 Pos	108	108	100	96.6, 100	108	100	96.6, 100

CI = Score Confidence Interval; Neg = Negative; Pos = Positive

Table 27: Aptima HPV 16 18/45 Genotype Assay Precision Study 3: Percentile Distribution of HPV 16 and HPV 18/45 Analyte S/CO Values

Description (copies/reaction)	HPV 16 S/CO				HPV 18/45 S/CO			
	Mean	2.5th	50th	97.5th	Mean	2.5th	50th	97.5th
HPV 16 IVT (60 copies) Low-Positive	3.71	2.81	3.76	4.33	0.02	0.00	0.00	0.40
HPV 18 IVT (85 copies) Low-Positive	0.30	0.02	0.29	0.57	5.26	4.64	5.23	6.00
HPV 45 IVT (60 copies) Low-Positive	0.36	0.10	0.35	0.66	4.63	3.81	4.57	5.55
HPV 18 IVT (85 copies) Low-Positive & HPV 45 IVT (60 copies) Low-Positive	0.40	0.05	0.40	0.84	7.85	6.90	7.82	8.92
HPV Negative (0 copies)	0.32	0.26	0.31	0.36	0.00	0.00	0.00	0.02
HPV 16 IVT (180 copies) Moderate-Positive	3.94	3.38	3.94	4.52	0.00	0.00	0.00	0.00
HPV 18 IVT (260 copies) Moderate-Positive	0.29	0.00	0.30	0.60	5.38	4.76	5.38	6.00
HPV 45 IVT (180 copies) Moderate-Positive	0.37	0.02	0.36	0.68	4.72	3.96	4.70	5.52
HPV 18 IVT (260 copies) Moderate-Positive & HPV 45 IVT (180 copies) Moderate-Positive	0.41	0.04	0.42	0.86	7.85	6.94	7.82	8.66

Table 28: Aptima HPV 18/45 Genotype Assay Precision Study 3: HPV 16 Analyte Signal Variability for Panel Members with an Expected Positive Result for HPV 16

Description (copies/reaction)	N	Mean S/CO	Inter-instrument		Inter-operator		Inter-lot		Inter-Run		Intra-Run		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
HPV 16 IVT (60 copies) Low-Positive	108	3.71	0.07	1.9	0.10	2.6	0.06	1.5	0.09	2.4	0.33	8.9	0.37	10.0
HPV 16 IVT (180 copies) Moderate-Positive	108	3.94	0.00	0.0	0.07	1.8	0.18	4.6	0.15	3.7	0.18	4.4	0.30	7.6

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 29: Aptima HPV 16 18/45 Genotype Assay Precision Study 3: HPV 18/45 Analyte Signal Variability for Panel Members with an Expected Positive Result for HPV 18/45

Description (copies/reaction)	N	Mean S/CO	Inter-instrument		Inter-operator		Inter-lot		Inter-Run		Intra-Run		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
HPV 18 IVT (85 copies) Low-Positive	108	5.26	0.00	0.0	0.00	0.0	0.03	0.6	0.13	2.5	0.32	6.0	0.35	6.6
HPV 45 IVT (60 copies) Low-Positive	108	4.63	0.11	2.3	0.04	0.9	0.46	10.0	0.00	0.0	0.30	6.4	0.56	12.1
HPV 18 IVT (85 copies) Low-Positive & HPV 45 IVT (60 copies) Low-Positive	108	7.85	0.00	0.0	0.13	1.7	0.32	4.1	0.24	3.1	0.41	5.2	0.59	7.5
HPV 18 IVT (260 copies) Moderate-Positive	108	5.38	0.00	0.0	0.00	0.0	0.08	1.4	0.07	1.3	0.28	5.3	0.30	5.6
HPV 45 IVT (180 copies) Moderate-Positive	108	4.72	0.13	2.7	0.06	1.3	0.51	10.7	0.08	1.6	0.16	3.4	0.56	11.9
HPV 18 IVT (260 copies) Moderate-Positive & HPV 45 IVT (180 copies) Moderate-Positive	108	7.85	0.00	0.0	0.05	0.6	0.27	3.4	0.18	2.2	0.34	4.3	0.47	6.0

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*

Cross-Reactivity

The analytical specificity of the Aptima HPV 16 18/45 Genotype Assay was evaluated with pools of residual ThinPrep liquid cytology specimens diluted 1:2.9 into STM (comparable to specimen transferred to an Aptima Transfer tube) and spiked with cultured bacteria, yeast, or fungi; cultured virus; or non-targeted HPV *in vitro* transcripts. The organisms and test concentrations for which no cross reactivity was observed are identified in Table 30. The study criteria for assessing the effect of the presence of microorganism on the specificity of the assay were based on positivity.

Table 30: 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		
Yeast/protozoa			
Candida albicans	$1x10^6$ CFU/mL	*Trichomonas vaginalis***	$1x10^5$ cells/mL

Table 30: Analytical Specificity Panel: Organisms and Concentration with No Cross-Reactivity *(continued)*

Organism	Test Concentration with No Cross-Reactivity	Organism	Test Concentration with No Cross-Reactivity
Viruses			
Adenovirus	$5.25x10^7$ PFU/mL	HIV-1	$2.5x10^6$ copies/mL
Cytomegalovirus	$1.58x10^6$ $TCID_{50}$/mL	Herpes simplex virus 1	$3.39x10^6$ $TCID_{50}$/mL
Epstein-Barr virus	$1.59x10^5$ TD_{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; IFU = Inclusion Forming Unit; 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 30, which was also spiked with a low concentration of HPV infected SiHa cells (1.6 cells/reaction) and HPV infected HeLa cells (0.3 cells/reaction). The study criteria for assessing the effect of the presence of microorganism on the sensitivity of the assay were based on positivity. The presence of the microorganisms did not interfere with the Aptima HPV 16 18/45 Genotype Assay with the exception of Trichomonas vaginalis (TV). Interference was observed with TV when present at concentrations greater than 3×10^4 cells/mL.

Interference

The substances described in Table 31 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 31: Substances Tested for Possible Interference with the Aptima HPV 16 18/45 Genotype Assay

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

*Concentration in the test sample; ThinPrep liquid cytology specimen diluted 1:2.9 into STM (comparable to specimen transferred to an Aptima Transfer tube)
**Personal 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 2000 System, the ThinPrep 5000 Processor, or the ThinPrep 5000 Processor with AutoLoader Operator's Manual.

Pre- and Post-cytology ThinPrep Liquid Cytology Samples Processed on ThinPrep 5000 Processor

Testing was conducted to determine the agreement of ThinPrep liquid cytology samples in PreservCyt Solution tested on the Aptima HPV 16 18/45 Genotype Assay before and after processing on the ThinPrep 5000 Processor. A total of 200 contrived ThinPrep liquid cytology samples (100 HPV positive, 100 HPV negative) were evaluated in the Aptima HPV 16 18/45 Genotype Assay before and after processing on the ThinPrep 5000 Processor. The study showed comparable performance between pre- and post-cytology samples at all concentrations tested. (Table 32)

Table 32: Pre- and Post-Cytology Sample Results

		Pre-cytology			
		Positive Samples (above C95)		Negative Samples (below C95)	
		Spiked with Hela at ~10X LoD (95% CI)	Spiked with Hela at 1.5-3X LoD (95% CI)	Spiked with Hela at 0.05X LoD (95% CI)	Un-spiked (95% CI)
Post -cytology	Positive Percent Agreement	100.0	97.5	N/A	N/A
		(83.9, 100.0)	(91.2, 99.3)		
		20/20	77/79		
	Negative Percent Agreement	N/A	0.0	100.0	100.0
			(0.0, 79.3)	(91.2, 100.0)	(94.0, 100.0)
			0/1	40/40	60/60
Total		20	80	40	60

CI = Confidence Interval

Panther System Expected Results: Prevalence of High-Risk HPV mRNA (CLEAR TRIAL)

The prevalence of high-risk HPV infection varies widely and is influenced by several factors, of which age is the greatest contributor.[19,20] Many studies have investigated HPV prevalence as determined by the detection of HPV DNA, however few studies report prevalence based on detection of HPV oncogenic mRNA. Women from a variety of clinical sites (n=18) representing a wide geographic distribution and a diverse population (10 states within the United States) were enrolled in a prospective clinical study known as the Clinical Evaluation of APTIMA mRNA (CLEAR) trial to evaluate the Aptima HPV assay, which detects 14 high-risk HPV types.[21] 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 33 for the atypical squamous cells of undetermined significance (ASC-US) and the negative for intraepithelial lesion or malignancy (NILM) populations.

Table 33: 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)

HR = High-risk; N/A = Not Applicable; 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 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.

Aptima HPV 16 18/45 Genotype Assay on the Panther System Clinical Study Design

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.[21]

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 from women in the ASC-US Study and in the NILM Study 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 by 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 status. They were also masked to 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 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.

Panther System Assay Performance

ASC-US ≥ 21 Years Population: Aptima HPV 16 18/45 Genotype Assay Clinical Performance

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 34.

Table 34: 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 35. 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 36.

Table 35: 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; Neg = Negative; Pos = Positive
*Women with Aptima HPV assay negative results were designated as Aptima HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

Table 36: 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	N/A (0/0)	N/A (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; N/A = Not Applicable; Neg = Negative; Pos = Positive
*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 37. 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 37: 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 38. 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 38: 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 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 at baseline 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 at baseline. 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 at baseline. An Aptima HPV assay negative result indicates that none of the 14 high-risk HPV types are present; women with Aptima HPV assay negative results 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 at baseline are presented in Table 39.

Table 39: 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; Neg = Negative; Pos = Positive
*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 at baseline (Table 40). Of the 10,348 women with Aptima HPV assay negative results from the original CLEAR trial, 9,799 had unverified disease status at baseline. 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 predict 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. This method differs from the one used to evaluate the Tigris DTS System, which imputed results from testing with the Aptima HPV assay and the Aptima HPV 16 18/45 Genotype Assay on the Tigris DTS System and not from testing on the Panther System. Therefore, it is not valid to make comparisons between verification-bias adjusted performance estimates in this section and those in the Tigris DTS System section. Both verification-bias adjusted performance estimates and unadjusted performance estimates based on the 808 women with verified disease status at baseline are presented.

Table 40: 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 41a. 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 at baseline are shown overall in Table 41b and by age group in Table 42.

Table 41a: 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; Neg = Negative; Pos = Positive
*Women with Aptima HPV assay negative results were designated as Aptima HPV 16 18/45 Genotype Assay negative for the purpose of analysis.
Verification-bias adjusted performance estimates for the Tigris DTS System section and the Panther System section use different imputation methods.

Table 41b: 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; Neg = Negative; Pos = Positive
*Women with Aptima HPV assay negative results were designated as Aptima HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

Table 42: 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; N/A Not Applicable; Neg = Negative; Pos = Positive
*Women with Aptima HPV assay negative results were designated as Aptima HPV 16 18/45 Genotype Assay negative for the purpose of analysis.

The relative risk of disease for Aptima 16 18/45 Genotype Assay positive versus negative outcomes at baseline are shown in Table 43 (verification-bias adjusted) and Table 44 (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 43: 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; Neg = Negative; Pos = Positive
*Women with Aptima HPV assay negative results were designated as Aptima HPV 16 18/45 Genotype Assay negative for the purpose of analysis.
Note: *Verification-bias adjusted performance estimates for the Tigris DTS System section and the Panther System section use different imputation methods.*

Table 44: 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; Neg = Negative; Pos = Positive
*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 16 18/45 Genotype Assay result are shown in Table 45 (verification-bias adjusted) and Table 46 (unadjusted). HPV types 16, 18, and/or 45 were 11.2 times more likely to be present in a woman with ≥CIN2 at baseline and 24.1 times more likely to be present in a woman with ≥CIN3 at baseline.

Table 45: 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.
Note: *Verification-bias adjusted performance estimates for the Tigris DTS System section and the Panther System section use different imputation methods.*

Table 46: 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 47.

Table 47: 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 48. The 3-year cumulative relative risk of disease for Aptima 16 18/45 Genotype Assay positive versus negative outcomes are shown in Table 49.

Table 48: 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 49: 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).

Panther System Assay Performance: Positivity Rate of High-Risk HPV mRNA (REACH Study for Primary Screening)

The positivity rate of high-risk HPV infection varies widely and is influenced by several factors, for which age is the greatest factor.[15,16] Many studies have investigated HPV positivity rate as determined by HPV DNA; however, few studies report *positivity rate* based on HPV oncogenic mRNA. As determined by the Aptima HPV Assay on the Panther System, the rate of HPV mRNA-positive results in the multicenter comparative retrospective real-world data (RWD) study known as the Real-World Evidence Evaluation of the Aptima HPV Assay for Cervical Health (REACH) study was categorized overall and by age group. Real-world data for the Aptima HPV cohort were obtained from 6 sites, which included integrated data networks, hospital systems, and large medical practices within the US representing a wide geographic distribution and a diverse population (6 states within the US). Based on the data from the REACH RWD study, HPV positivity rate by Aptima HPV Assay overall and by age group is shown in Table 50.

Table 50: Summary of HPV Positivity Rate by Aptima HPV Assay Result by Age Group in the REACH Study Population

	Positivity Rate % (x/n)
Overall	7.7 (36,385 / 470,468)
Age Group (years)	
25-29	35.4 (6,341 / 17,919)
30-39	10.0 (14,927 / 149,981)
40-49	5.9 (6,928 / 116,729)
50-64	4.4 (7,071 / 159,785)
≥65	4.3 (1,118 / 26,054)

Aptima HPV Assay and Aptima HPV 16 18/45 Genotype Assay on the Panther System: Study Design (REACH Study for Primary Screening)

Use of the Aptima HPV Assay on the Panther System as a primary cervical cancer screening test (primary screening) was evaluated using data collected in the US in a large retrospective, multicenter RWD study, known as the REACH study.

The REACH study was conducted to evaluate the clinical performance of the Aptima HPV Assay compared to an FDA-approved HPV DNA test for primary screening. Data for the Aptima and FDA-approved HPV DNA test (comparator cohort) cohorts were obtained from 8 sites (5 sites for Aptima, 2 sites for comparator cohort, 1 site that included both) which included integrated data networks (IDNs), hospital systems, and large medical practices representing a wide geographic distribution and a diverse enrollment population (7 states within the US).

Women ≥25 years of age, who underwent cervical cancer screening with an HPV assay result and liquid-based cytology in routine clinical practice were assessed for eligibility. Women were excluded if they had a known history of cervical biopsy, cytology and/or HPV test of any result within 300 days prior to the index HPV test (the first HPV test result for a patient that meets study eligibility); cervical cancer, ablative or excisional treatment or surgery to the cervix, and/or hysterectomy prior to or on the date of the index HPV test. Any woman with a histology result available within 0 to 6 days of the index HPV test was excluded.

All data were extracted in a retrospective manner from information routinely collected in the electronic medical records. To ensure that the disease risk/prevalence between Aptima HPV and comparator cohorts was comparable, the estimates were adjusted for cytology and the analyses were performed for two age populations (25 – 29 years and 30 years and older).

The study's outcome was histology confirmed cervical disease defined as ≥CIN3 and ≥CIN2.

Determination of Disease Status

Cervical disease status (≥CIN2 or ≥CIN3) at baseline was determined by the most severe histology result available between 7 days and 12 weeks (84 days) of the index (first valid) HPV test result. Cervical disease status (<CIN2) at baseline was determined by either colposcopic evaluation not requiring histology or histology result (<CIN2) available between 7 days and 12 weeks (84 days) of the index (first valid) HPV test result.

Multiple imputation was performed for women with a positive index HPV test and missing cervical disease status during this time period.

Analyses

In the REACH study, the performance of the Aptima HPV Assay was evaluated based on the ratios of clinical sensitivities (ratios of true positive rates) and the ratios of false positive rates (**Kondratovich 2008**) between the Aptima HPV Assay and the FDA-approved HPV DNA test for both ≥CIN3 and ≥CIN2 clinical outcomes. For both the Aptima HPV Assay and the FDA-approved HPV DNA test, a woman was considered as having a positive primary screening result as shown in Table 51.

Table 51: Determination of a Positive Primary Screening Result

		Cytology Result			
		>ASC-US	**ASC-US**	**UNSAT**	**NILM**
Aptima HPV Test Result	**HPV 16/18 45**	Positive	Positive	Positive	Positive
	11 Other	Positive	Positive	Positive	Negative
	Negative	Negative	Negative	Negative	Negative
FDA-approved HPV DNA Test Result	**HPV 16/18**	Positive	Positive	Positive	Positive
	12 Other	Positive	Positive	Positive	Negative
	Negative	Negative	Negative	Negative	Negative

For women with the Aptima HPV Assay positive results and where genotyping was required, an Aptima HPV 16 18/45 Genotype Assay result was used to determine HPV genotype (categorized as HPV 16, HPV 18/45, or 11 other high-risk HPV types). The two-sided 95% confidence intervals for the ratios of clinical sensitivities and the ratios of false positive rates were calculated using the bootstrap method. Analyses were performed for the two age populations (25 – 29 years and 30 years and older) adjusted for cytology.

For women with Aptima HPV 16 18/45 Genotype Assay results available, descriptive summaries are presented by cohort below.

Panther System Assay Performance (REACH Study for Primary Screening)

Primary Screening Population: Aptima HPV Assay Clinical Performance and Aptima HPV 16 18/45 Genotype Assay Descriptive Summaries

The number of women assessed for eligibility was 543,233 for the Aptima HPV cohort and 223,596 for the comparator cohort (data not shown). In total, 470,468 and 190,053 women in the Aptima HPV cohort and the comparator cohort, respectively, who were 25 years of age and older were evaluable and enrolled in the REACH study (see Table 52).

Table 52: Demographics and Characteristics by Cohort

	AHPV Cohort (N=470,468)	Comparator Cohort (N=190,053)
Age Group (years)		
25-29	17,919 (3.8%)	8,781 (4.6%)
30-39	149,981 (31.9%)	72,520 (38.2)
40-49	116,729 (24.8%)	44,788 (23.6%)
50-64	159,785 (34.0%)	57,491 (30.2%)
≥65	26,054 (5.5%)	6,473 (3.4%)
Race		
African American	52,376 (11.1%)	16,588 (8.7%)
Asian	27,072 (5.8%)	6,432 (3.4%)
White	317,389 (67.5%)	155,733 (81.9%)
Other	28,655 (6.1%)	6,335 (3.3%)
Unknown/Not reported	44,976 (9.6%)	4,965 (2.6%)
Ethnicity		
Hispanic or Latino	44,510 (9.5%)	7,148 (3.8%)
Not Hispanic or Latino	373,310 (79.3%)	178,822 (94.1%)
Unknown/Not reported	52,648 (11.2%)	4,083 (2.1%)
HPV Vaccination Status		
Vaccinated	17,499 (3.7%)	6,843 (3.6%)
Not Vaccinated/Unknown	452,969 (96.3%)	183,210 (96.4%)
Cytology Results		
Unsatisfactory	5,666 (1.2%)	1,891 (1.0%)
NILM	429,002 (91.2%)	167,314 (88.0%)
ASC-US	27,807 (5.9%)	15,478 (8.1%)
>ASC-US	7,993 (1.7%)	5,370 (2.8%)

AHPV = Aptima HPV
ASC-US = Atypical squamous cells of undetermined significance
NILM = Negative for intraepithelial lesion or malignancy

The HPV positivity rate for the Aptima HPV cohort and the comparison cohort was 7.7% and 9.0%, respectively (Table 53).

Table 53: HPV Positivity Overall and by Age Group for Each Cohort

	Positivity Rate % (x/n)	
	Study Cohort	
	AHPV Cohort (N=470,468)	Comparator Cohort (N=190,053)
Overall	7.7 (36,385 / 470,468)	9.0 (17,118 / 190,053)
Age Group (years)		
25-29	35.4 (6,341 / 17,919)	32.4 (2,848 / 8,781)
30-39	10.0 (14,927 / 149,981)	10.8 (7,850 / 72,520)
40-49	5.9 (6,928 / 116,729)	7.0 (3,135 / 44,788)
50-64	4.4 (7,071 / 159,785)	5.1 (2,937 / 57,491)
≥65	4.3 (1,118 / 26,054)	5.4 (348 / 6,473)

AHPV = Aptima HPV

The results of the Aptima HPV Assay and FDA-approved HPV DNA test by disease status for each age cohort among women with colposcopy performed are presented in Table 54. The prevalence of ≥CIN3 in the Aptima HPV and comparator cohorts was 0.3% (1,340/470,468) and 0.5% (886/190,053), respectively, and the prevalence of ≥CIN2 was 0.6% (2,617/ 470,468) and 0.7% (1,376/190,053), respectively.

Table 54: HPV Assay Results by Disease Status for Each Cohort Among Women With Colposcopy Performed

Age Group	Study Cohort	HPV Assay Result	Colposcopy No Biopsy	Normal	CIN1	CIN2	CIN3	AIS	Cancer	Total
25-29	AHPV	Positive	260	1,352	1,116	356	217	7	2	3,310
		Negative	63	119	37	7	1	0	0	227
		Total	323	1,471	1,153	363	218	7	2	3,537
	Comparator	Positive	152	398	663	114	150	3	3	1,483
		Negative	42	52	56	7	8	0	0	165
		Total	194	450	719	121	158	3	3	1,648
≥30	AHPV	Positive	682	5,390	2,665	890	1,004	48	32	10,711
		Negative	389	2,004	322	24	25	1	3	2,768
		Total	1071	7,394	2,987	914	1,029	49	35	13,479
	Comparator	Positive	492	1,813	2,003	355	635	30	29	5,357
		Negative	263	941	226	14	21	0	7	1,472
		Total	755	2,754	2,229	369	656	30	36	6,829

AHPV = Aptima HPV
AIS = adenocarcinoma in situ
CIN2 = cervical intraepithelial neoplasia grade 2
CIN3 = cervical intraepithelial neoplasia grade 3

Table 55 and 56 show the results by age group for the detection of ≥CIN3 and ≥CIN2. For the detection of ≥CIN3 after adjustment by cytology, the ratios of clinical sensitivities between the Aptima HPV Assay and FDA-approved HPV DNA test in the 25-29 and ≥30 age groups were 0.9693 and 0.9957, respectively. For the detection of ≥CIN2 after adjusting for cytology, the ratios of clinical sensitivities between the Aptima HPV Assay and FDA-approved HPV DNA test in the 25-29 and ≥30 age groups were 1.3277 and 1.1851, respectively. These results demonstrate that the sensitivity of the Aptima HPV Assay is clinically comparable to the FDA-approved HPV DNA test and the Aptima HPV Assay is similarly effective for use in primary screening for the detection of ≥CIN3 and ≥CIN2.

Table 55: Performance of Aptima HPV Assay Compared to FDA-approved HPV DNA *Test for Detection of ≥CIN3 and ≥CIN2 in Women 30 Years and Older*

	Parameter	Point Estimate[1]	Lower Bound of 95% CI[2]	Upper Bound of 95% CI[2]
≥CIN3	Ratio of Sensitivities	0.9957	0.9162	1.0961
	Ratio of False Positive Rates	1.2105	1.1793	1.2418
≥CIN2	Ratio of Sensitivities	1.1851	1.1043	1.2754
	Ratio of False Positive Rates	1.1816	1.1454	1.2194

CI = confidence interval
CIN2 = cervical intraepithelial neoplasia grade 2
CIN3 = cervical intraepithelial neoplasia grade 3
[1]Adjusted for cytology result
[2]Confidence intervals were calculated by the bootstrap method.

Table 56: Performance of Aptima HPV Assay Compared to FDA-approved HPV DNA Test for Detection of ≥CIN3 and ≥CIN2 in Women 25-29 Years Old

	Parameter	Point Estimate[1]	Lower Bound of 95% CI[2]	Upper Bound of 95% CI[2]
≥CIN3	Ratio of Sensitivities	0.9693	0.7011	1.2652
	Ratio of False Positive Rates	1.1191	1.0485	1.2501
≥CIN2	Ratio of Sensitivities	1.3277	1.0992	1.6138
	Ratio of False Positive Rates	1.0436	0.9669	1.1846

CI = confidence interval
CIN2 = cervical intraepithelial neoplasia grade 2
CIN3 = cervical intraepithelial neoplasia grade 3
[1]Adjusted for cytology result
[2]Confidence intervals were calculated by the bootstrap method.

Table 57 displays the descriptive summary of the Aptima HPV 16 18/45 Genotype Assay results. In the Aptima HPV cohort, 36,385 women had positive HPV results. In the comparator cohort, 17,118 women had positive HPV results. Of those women with positive HPV results, the Aptima HPV and comparator cohorts compare as follows: 6.9% and 19.7% were genotype positive, 35.8% and 76.4% were genotype negative, with 57.3% and 3.9% unknown/not reported, respectively.

Table 57: Aptima HPV 16 18/45 Genotype Assay REACH Study Descriptive Summary

		AHPV Cohort (N=470,468)	Comparator Cohort (N=190,053)	Total (N=660,521)
HPV Genotype Assay/Test Result among Women with Positive HPV Assay/Test Result	16 positive only	1,362 (3.7%)	2,449(14.3%)	3,811 (7.1%)
	18 positive only	0 (0.0%)	834 (4.9%)	834 (1.6%)
	18/45 positive only	1,140 (3.1%)	0 (0.0%)	1,140 (2.1%)
	16 and 18 positives	0 (0.0%)	86 (0.5%)	86 (0.2%)
	16 and 18/45 positives	40 (0.1%)	0 (0.0%)	40 (0.1%)
	GT Negative	13,012 (35.8%)	13,077 (76.4%)	26,089 (48.8%)
	Unknown/not reported	20,831(57.3%)	672 (3.9%)	21,503 (40.2%)
	Not applicable	434,083	172,935	607,018

AHPV = Aptima HPV
GT = genotype

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

Samples Processed on the ThinPrep 5000 Processor

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 on the ThinPrep 5000 Processor, 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 58 for the total population, in Table 59 for the NILM (≥30 years) population, and in Table 60 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 58: 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 Processed on the ThinPrep 5000 Processor

		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 valid 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 all samples with discordant results for Samples A and B.

Table 59: 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 Processed on the ThinPrep 5000 Processor

		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 valid 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 all samples with discordant results for Samples A and B.

Table 60: 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 Processed on the ThinPrep 5000 Processor

		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 valid 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 all samples with discordant results for Samples A and B.

Samples Processed on the ThinPrep Genesis Processor

A study was conducted to assess the agreement of Aptima HPV 16 18/45 Genotype Assay results on the Panther System between cervical samples tested prior to (Pre-cytology) and after (Post-cytology) cytology processing on the ThinPrep Genesis 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 on the ThinPrep Genesis Processor, one 1-mL of the residual ThinPrep specimen was manually transferred into an Aptima Specimen Transfer tube (Post-cytology sample C).

A total of 184 samples with positive Aptima HPV assay results were evaluated using the Aptima HPV 16 18/45 Genotype Assay. The frequency results of this study are shown in Table 61 for the total population, in Table 62 for the NILM (≥30 years) population, and in Table 63 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 61: Total Population[1]: HPV 16 and/or 18/45 Genotypes Detected by the Aptima HPV 16 18/45 Genotype Assay in Pre- and Post-cytology Samples Processed on the ThinPrep Genesis Processor

		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**	16	0	1	0
	HPV 16 Neg, HPV 18/45 Pos	0	9	0	0
	Other HR HPV Pos, HPV[3] 16/18/45 Neg	0	2	153	3

HR = high-risk, Neg = negative, Pos = positive.
[1] Total population includes >ASC-US, ASC-US, NILM
[2] All samples have a complete set of valid 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 all samples with discordant results for Samples A and B.

Table 62: NILM≥30 Years Population: HPV 16 and/or 18/45 Genotypes Detected by the Aptima HPV 16 18/45 Genotype Assay in Pre- and Post-cytology Samples Processed on the ThinPrep Genesis Processor

		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**	4	0	1	0
	HPV 16 Neg, HPV 18/45 Pos	0	7	0	0
	Other HR HPV[2] Pos, HPV 16/18/45 Neg	0	2	63	2

HR = high-risk, Neg = negative, Pos = positive.
[1] All samples have a complete set of valid 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 all samples with discordant results for Samples A and B.

Table 63: ASC-US ≥21 Years Population: HPV 16 and/or 18/45 Genotypes Detected by the Aptima HPV 16 18/45 Genotype Assay in Pre- and Post-cytology Samples Processed on the ThinPrep Genesis Processor

		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	1	0	0
	Other HR HPV[2] Pos, HPV 16/18/45 Neg	0	0	55	1

HR = high-risk, Neg = negative, Pos = positive.
[1] All samples have a complete set of valid 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 all samples with discordant results for Samples A and B.

In addition to the above studies performed on clinical specimens, contrived ThinPrep vials prepared with individual HPV negative clinical matrix were used to generate manual pre-cytology aliquots and manual post-cytology aliquots for testing with Aptima HPV 16 18/45 Genotype Assay. A total of 60 positive ThinPrep vials, including 30 HPV 16 positive (20 vials at 1.5xLoD and 10 vials at 4xLoD), 15 HPV 18 positive (10 vials at 1.5xLoD and 5 vials at 4xLoD) and 15 HPV 45 positive (10 vials at 1.5xLoD and 5 vials at 4xLoD), were prepared. Two pre-cytology aliquots, A and B, were removed manually and one post-cytology aliquot was removed manually after the cytology process by ThinPrep Genesis Processor. The results from this study are presented in Table 64.

Table 64: Detection of HPV 16 and/or 18/45 Genotypes by the Aptima HPV 16 18/45 Genotype Assay in Pre- and Post-cytology Contrived Samples Processed on the ThinPrep Genesis Processor

		Pre-cytology Samples A and B			
		HPV 16 Pos, HPV 18/45 Neg	HPV 16 Neg, HPV 18/45 Pos	HPV 16/18/45 Neg	Undetermined*
Post-cytology Sample C	HPV 16 Pos, HPV 18/45 Neg	24	0	0	1
	HPV 16 Neg, HPV 18/45 Pos	0	26	1	2
	HPV 16/18/45 Neg	5	2	29	0
Total		29	28	30	3
Percent Agreement 95% (CI)		82.8% (24/29) 65.5 - 92.4%	92.9% (26/28) 77.4 - 98.0%	96.7% (29/30) 83.3 - 99.4%	N/A

*Includes all samples with discordant results between samples A and B.

Agreement with Reverse Transcription-PCR Sequencing

The analytical performance of the Aptima HPV 16 18/45 Genotype Assay for detection of target was assessed against an in-house validated reverse transcription-polymerase chain reaction (RT-PCR) sequencing test specific for E6/E7 mRNA from the same 14 high risk HPV types detected by the Aptima HPV assay. Sequencing was performed by an external commercial laboratory.

Cervical specimens collected at baseline from the ASC-US and NILM populations of the CLEAR trial from women with Aptima HPV assay positive results were tested with the RT-PCR sequencing test and compared to the Aptima HPV 16 18/45 Genotype Assay results. In total, 842 samples were tested: 359 from the ASC-US population and 483 from the NILM population.

For the ASC-US and NILM populations, Aptima HPV 16 18/45 Genotype Assay results by RT-PCR sequencing test results are shown in Table 65a and Table 65b, respectively. Positive and negative percent agreements for the ASC-US and NILM populations are shown in Table 65c and Table 65d, respectively.

Table 65a: ASC-US ≥ 21 Years Population: Comparison of Aptima HPV 16 18/45 Genotype Assay and RT-PCR Sequencing Test Results Including Only Samples With Aptima HPV Assay Positive Results

		RT-PCR Sequencing Test Results										
		One HR Type				Two HR Types				>2 HR Types		
Aptima HPV-GT Assay Result	No HR Type	16	18	45	Other HR	16 & Other	18 & Other	45 & Other	2 Other HR	≥1 of 16/18/45 Present	Only Other HR Present	Ind
16+, 18/45-	26	27	0	0	6	8	0	0	1	3	0	0
16-, 18/45+	6	0	16	8	5	0	5	3	1	4	0	0
16+, 18/45+	0	0	1	0	0	1	0	0	0	1	0	0
16-, 18/45-	82	0	0	1	127	0	1	2	16	0	6	2
Total	114	27	17	9	138	9	6	5	18	8	6	2

+ = Positive; - = Negative; HPV-GT = Aptima HPV 16 18/45 Genotype Assay; HR = high risk; Ind = indeterminate, unable to determine positivity for types 16/18/45 due to invalid RT-PCR sequencing test results.

Note: *Columns with all zeros are not shown*

Table 65b: NILM ≥ 30 Years Population: Comparison of Aptima HPV 16 18/45 Genotype Assay and RT-PCR Sequencing Test Results Including Only Samples With Aptima HPV Assay Positive Results

		RT-PCR Sequencing Test Results									
		One HR Type				Two HR Types				>2 HR Types	
Aptima HPV-GT Assay Result	No HR Type	16	18	45	Other HR	16 & Other	18 & Other	45 & Other	2 Other HR	≥ 1 of 16/18/45 Present	Ind
16+, 18/45-	27	19	0	0	2	1	0	0	0	0	0
16-, 18/45+	7	0	20	13	1	0	2	5	0	1	0
16+, 18/45+	0	0	0	0	1	0	0	0	0	0	0
16-, 18/45-	226	0	0	3	148	1	0	0	4	0	2
Total	260	19	20	16	152	2	2	5	4	1	2

+ = Positive; - = Negative; HPV-GT = Aptima HPV 16 18/45 Genotype Assay; HR = high risk; Ind = indeterminate, unable to determine positivity for types 16/18/45 due to invalid RT-PCR sequencing test results.

Note: *Columns with all zeros are not shown*

Table 65c: ASC-US ≥ 21 Years Population: Comparison of Aptima HPV 16 18/45 Genotype Assay and RT-PCR Sequencing Test Results Including Only Samples With Aptima HPV Assay Positive Results

Aptima HPV-GT Assay Result	RT-PCR Sequencing Test Results		
		16/18/45-	
	16/18/45+	Other HR+	HR-
16/18/45+	77	13	32
16/18/45-	4	149	82
Total	81	162	114
Positive Percent Agreement: 95.1 (77/81) (95% CI: 88.0, 98.1)			
Negative Percent Agreement: 92.0 (149/162) (95% CI: 86.8, 95.3)			

+ = Positive; - = Negative; CI = Confidence Interval; HPV-GT = Aptima HPV 16 18/45 Genotype Assay; HR = high risk

Table 65d: NILM ≥ 30 Years Population: Comparison of Aptima HPV 16 18/45 Genotype Assay and RT-PCR Sequencing Test Results Including Only Samples With Aptima HPV Assay Positive Results

Aptima HPV-GT Assay Result	RT-PCR Sequencing Test Results		
		16/18/45-	
	16/18/45+	Other HR+	HR-
16/18/45+	61	4	34
16/18/45-	4	152	226
Total	65	156	260
Positive Percent Agreement: 93.8 (61/65) (95% CI: 85.2, 97.6)			
Negative Percent Agreement: 97.4 (152/156) (95% CI: 93.6, 99.0)			

+ = Positive; - = Negative; CI = Confidence Interval; HPV-GT = Aptima HPV 16 18/45 Genotype Assay; HR = high risk

Clinical Cutoff Determination for the Aptima HPV 16 18/45 Genotype Assay

The method used to establish the clinical cutoff for detecting high grade cervical disease (≥CIN2) for the Aptima HPV 16 18/45 Genotype Assay is described in the *Clinical Cutoff Determination for the Aptima HPV 16 18/45 Genotype Assay* in the Tigris DTS System section. The cutoff for the Aptima HPV 16 18/45 Genotype Assay was set at 1.00 S/CO for both HPV 16 and HPV 18/45.

Limit of Detection at the Clinical Cutoff

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 determined by testing dilution panels of *in vitro* transcripts (IVT) for all genotypes 16, 18 and 45 and 3 HPV-infected cell lines: SiHa, HeLa and MS751 (ATCC, Manassas, Virginia). For the IVT panels, specimen transport media was spiked with IVT at various concentrations and then diluted with individual negative ThinPrep liquid cytology specimens prior to testing. For the HPV-infected cell panels, pools of HPV-negative ThinPrep liquid cytology specimens were spiked with HPV-infected cells at various concentrations and then diluted with specimen transport media prior to testing. 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 and concentration tested in each run. The 95% detection limit (Table 66) was calculated from Probit regression analysis of the positivity results for each dilutional panel.

Table 66: 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 copies/reaction (19.1, 30.9)
HPV 18	26.1 copies/reaction (21.2, 33.9)
HPV 45	34.5 copies/reaction (28.5, 43.6)
SiHa	0.42 cells/reaction (0.30, 0.72)
HeLa	0.66 cells/reaction (0.40, 1.40)
MS751	0.17 cells/reaction (0.13, 0.26)

CI = Confidence Interval

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 diluted with STM 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 67a and Table 67b, along with a summary of the agreement with expected results for HPV 16 and HPV 18/45 respectively. Table 68 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 69 for Study 1 and Table 70 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 71 for Study 1 and Table 72 for Study 2 for the panel members with an expected positive result for HPV 18/45.

Table 67a: 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)
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 = Confidence Interval

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

Table 67b: 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)	HPV 18/45 Expected Result	Percent Agreement (95% CI)	
		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)
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 = Confidence Interval
Note: *The percent agreement may have been affected by variations in spiking, diluting, and/or aliquoting.*

Table 68: 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 69: 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 70: 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 71: 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 72: 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.*

Reproducibility of Aptima HPV 16 18/45 Genotype Assay on Pre-cytology Aliquot Removed by ThinPrep Genesis Processor

Reproducibility of the Aptima HPV 16 18/45 Genotype Assay on pre-cytology aliquots removed by the ThinPrep Genesis Processor was evaluated by testing the panel members listed in the following table. All panel members were prepared in PreservCyt medium containing 10^5 cells/mL of C33A (non-HPV) cells as background. Negative panel members were not spiked with any HPV cell lines. Positive panel members were prepared by spiking HPV 16 or HPV 18 positive cells at the indicated concentrations. The performance with the pre-cytology aliquot removed by ThinPrep Genesis Processor was compared to the performance with the manual pre-cytology aliquot.

Table 73: Panel Members Used For Testing Pre-Cytology Aliquots Removed by the ThinPrep Genesis Processor with the Aptima HPV 16 18/45 Genotype Assay

Panel Member	Cell Line	Concentration
Negative	N/A	N/A
Low Positive 1	HeLa (HPV 18)	3x LoD[a]
Moderate Positive 1	HeLa (HPV 18)	5x LoD[a]
Low Positive 2	SiHa (HPV 16)	3x LoD[b]
Moderate Positive 2	SiHa (HPV 16)	5x LoD[b]

[a]LoD of the AHPV assay at the clinical cutoff for Hela cell line is 0.11 cells/reaction.

[b]LoD of the AHPV assay at the clinical cutoff for SiHa cell line is 0.25 cells/reaction.

For each panel member, manual pre-cytology aliquots were prepared by three operators. In addition, pre-cytology aliquots were prepared by three ThinPrep Genesis Processors using "Aliquot" process. Each ThinPrep Genesis Processor or operator prepared three replicate aliquots per run, and two runs per day across five days. For each panel member, a total of 90 aliquots were prepared for manual pre-cytology and a total of 90 pre-cytology aliquots were prepared by ThinPrep Genesis Processor. The Aptima HPV 16 18/45 Genotype Assay was performed on the Panther System for all study aliquots. The percent agreement with expected results, analyte S/CO percentile as well as variance analysis are presented in the following tables.

Table 74: HPV 16 Result Agreement and Percentile Distribution of Analyte S/CO Values, Automated Pre-Cytology Aliquot Vs. Manual Pre-Cytology Aliquot

Panel	Expected Result	Aliquot	N (Total)	n (Expected)	Percent agreement (95% CI)	Analyte S/CO Percentile 5th	50th	95th
Negative	Neg	Manual	90	90	100% (95.9-100%)	0.29	0.31	0.34
		Genesis	90	90	100% (95.9-100%)	0.29	0.31	0.33
Low Positive 1	Neg	Manual	90	90	100% (95.9-100%)	0.07	0.33	0.44
		Genesis	90	90	100% (95.9-100%)	0.11	0.34	0.48
Low Positive 2	Pos	Manual	90	90	100% (95.9-100%)	2.04	2.83	3.21
		Genesis	90	90	100% (95.9-100%)	2.04	2.86	3.23
Moderate Positive 1	Neg	Manual	90	90	100% (95.9-100%)	0.08	0.30	0.48
		Genesis	90	90	100% (95.9-100%)	0.05	0.34	0.49
Moderate Positive 2	Pos	Manual	90	90	100% (95.9-100%)	2.79	3.09	3.37
		Genesis	90	90	100% (95.9-100%)	2.71	3.10	3.3

Table 75: HPV 18/45 Result Agreement and Percentile Distribution of Analyte S/CO Values, Automated Pre-Cytology Aliquot Vs. Manual Pre-Cytology Aliquot

Panel	Expected Result	Aliquot	N (Total)	n (Expected)	Percent agreement (95% CI)	Analyte S/CO Percentile		
						5th	50th	95th
Negative	Neg	Manual	90	90	100% (95.9-100%)	0	0	0.02
		Genesis	90	90	100% (95.9-100%)	0	0	0.01
Low Positive 1	Pos	Manual	90	89	98.9% (94.0-99.8)	1.80	3.57	4.82
		Genesis	90	87	96.7% (90.7-98.9)	1.20	3.60	4.89
Low Positive 2	Neg	Manual	90	90	100% (95.9-100%)	0	0	0
		Genesis	90	90	100% (95.9-100%)	0	0	0
Moderate Positive 1	Pos	Manual	90	90	100% (95.9-100%)	3.05	4.37	5.36
		Genesis	90	90	100% (95.9-100%)	3.50	4.43	5.38
Modearte Positive 2	Neg	Manual	90	90	100% (95.9-100%)	0	0	0
		Genesis	90	90	100% (95.9-100%)	0	0	0

Table 76a: Variance Analysis - HPV 16 Positive Panel Members, Automated Pre-Cytology Aliquot Vs. Manual Pre-Cytology Aliquot

Reproducibility - HPV-GT Assay for HPV 16													
Sample Pool	System	N	Mean S/CO	Within run		Between run		Between day		Between Operator/ Instrument		Total Reproducibility	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Positive 2	Genesis	90	2.8	0.3	12.0%	0.2	5.6%	0.0	0.0%	0.1	4.5%	0.4	14.0%
	Manual	90	2.8	0.4	13.2%	0.1	2.7%	0.2	6.2%	0.0	0.0%	0.4	14.8%
Moderate Positive 2	Genesis	90	3.1	0.2	6.2%	0.0	0.0%	0.0	0.0%	0.0	1.5%	0.2	6.4%
	Manual	90	3.1	0.2	5.8%	0.0	0.0%	0.0	1.2%	0.0	0.5%	0.2	5.9%

Table 76b: Variance Analysis - HPV 18/45 Positive Panel Members, Automated Pre-Cytology Aliquot Vs. Manual Pre-Cytology Aliquot

Reproducibility - HPV-GT Assay for HPV 18/45													
Sample Pool	System	N	Mean S/CO	Within run		Between run		Between day		Between Operator/ Instrument		Total Reproducibility	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Positive 1	Genesis	90	3.4	1.0	29.3%	0.4	11.4%	0.2	7.2%	0.0	0.0%	1.1	32.2%
	Manual	90	3.4	0.9	28.0%	0.0	0.0%	0.4	12.6%	0.0	0.0%	1.0	30.7%
Moderate Positive 1	Genesis	90	4.4	0.5	11.8%	0.1	3.0%	0.4	8.6%	0.0	0.0%	0.7	14.9%
	Manual	90	4.3	0.6	14.5%	0	0.0%	0.3	7.2%	0.2	3.5%	0.7	16.5%

Reproducibility of Aptima HPV 16 18/45 Genotype Assay on Post-cytology Aliquot Removed by ThinPrep Genesis Processor

Reproducibility of the Aptima HPV 16 18/45 Genotype Assay on post-cytology aliquots removed by the ThinPrep Genesis Processor was evaluated by testing the panel members listed in the following table. All panel members were prepared in PreservCyt medium containing 10^5 cells/mL of C33A (non-HPV) cells as background. Negative panel members were not spiked with any HPV cell lines. Positive panel members were prepared by spiking HPV 16 or HPV 18 positive cells at the indicated concentrations.

Table 77: Panel Members Used For Testing Post-Cytology Aliquots Removed by the ThinPrep Genesis Processor with the Aptima HPV 16 18/45 Genotype Assay

Panel Member	Cell Line	Concentration
Negative	N/A	N/A
HPV-16 Low Positive 1	SiHa	0.0495 cells/reaction
HPV-16 Low Positive 2	SiHa	1.98 cells/reaction
HPV-16 Moderate Positive	SiHa	4.95 cells/reaction
HPV-18 Low Positive 1	HeLa	0.01485 cells/reaction
HPV-18 Low Positive 2	HeLa	0.594 cells/reaction
HPV-18 Moderate Positive	HeLa	1.485 cells/reaction

This study was conducted using manual pre-cytology aliquots and post-cytology aliquots prepared by the ThinPrep Genesis Processor. Two runs were conducted on each of three ThinPrep Genesis Processors per day across three days. For each run, a manual aliquot was taken from each ThinPrep vial prior to slide processing on the ThinPrep Genesis Processor. Subsequently, a ThinPrep slide was prepared from the vial using the "Slide" process on ThinPrep Genesis Processor, followed by a post-cytology aliquot prepared from the same vial using the "Aliquot" process on ThinPrep Genesis Processor. All prepared aliquots were tested with the Aptima HPV assay and only samples with positive Aptima HPV results were reflex tested in one replicate in the Aptima HPV 16 18/45 Genotype Assay on the Panther System.

The percent agreement, percentile distribution of S/CO values as well as S/CO variance analysis are presented below.

Table 78: HPV 16 Agreement and Percentile Distribution of Analyte S/CO Values for All Panel Members

Panel Member	Expected Result	Aliquot	N (Total)	n (Expected)	Percent agreement (95% CI) (HPV 16)	Analyte S/CO Percentile (HPV 16)		
						5th	50th	95th
HPV 16 Low positive 1	Pos	Pre-cytology	4	4	100% (59.7% - 100%)	1	1.29	2.42
		Post-cytology	1	1	100% (27.0% - 100%)	1.18	1.18	1.18
HPV 16 Low positive 2	Pos	Pre-cytology	14	14	100% (83.8% - 100%)	2.45	3.06	3.3
		Post-cytology	18	18	100% (86.9% - 100%)	2.52	3.11	3.33

Table 78: HPV 16 Agreement and Percentile Distribution of Analyte S/CO Values for All Panel Members *(continued)*

Panel Member	Expected Result	Aliquot	N (Total)	n (Expected)	Percent agreement (95% CI) (HPV 16)	Analyte S/CO Percentile (HPV 16)		
						5th	50th	95th
HPV 16 Moderate Positive	Pos	Pre-cytology	18	18	100% (86.9% - 100%)	2.9	3.12	3.44
		Post-cytology	18	18	100% (86.9% - 100%)	2.61	3.19	3.41
HPV 18 Low positive 1	Neg	Pre-cytology	2	2	100% (42.5% - 100%)	0.34	0.35	0.35
		Post-cytology	6	6	100% (68.9% - 100%)	0.22	0.35	0.54
HPV 18 Low positive 2	Neg	Pre-cytology	18	18	100% (86.9% - 100%)	0	0.3	0.59
		Post-cytology	18	18	100% (86.9% - 100%)	0.04	0.26	0.56
HPV 18 Moderate Positive	Neg	Pre-cytology	18	18	100% (86.9% - 100%)	0.12	0.35	0.59
		Post-cytology	18	18	100% (86.9% - 100%)	0.05	0.28	0.59

Table 79: HPV 18/45 Agreement and Percentile Distribution of Analyte S/CO Values for All Panel Members

Panel Member	Expected Result	Aliquot	N (Total)	n (Expected)	Percent agreement (95% CI) (HPV 18/45)	Analyte S/CO Percentile (HPV 18/45)		
						5th	50th	95th
HPV 16 Low positive 1	Neg	Pre-cytology	4	4	100% (59.7% - 100%)	0	0	0
		Post-cytology	1	1	100% (27.0% - 100%)	0	0	0
HPV 16 Low positive 2	Neg	Pre-cytology	14	14	100% (83.8% - 100%)	0	0	0
		Post-cytology	18	18	100% (86.9% - 100%)	0	0	0
HPV 16 Moderate Positive	Neg	Pre-cytology	18	18	100% (86.9% - 100%)	0	0	0
		Post-cytology	18	18	100% (86.9% - 100%)	0	0	0
HPV 18 Low positive 1	Pos	Pre-cytology	2	2	100% (42.5% - 100%)	1.79	2.38	2.96
		Post-cytology	6	4	66.7% (30.0% - 90.3%)	0.47	3.01	4.06

Table 79: HPV 18/45 Agreement and Percentile Distribution of Analyte S/CO Values for All Panel Members *(continued)*

Panel Member	Expected Result	Aliquot	N (Total)	n (Expected)	Percent agreement (95% CI) (HPV 18/45)	Analyte S/CO Percentile (HPV 18/45)		
						5th	50th	95th
HPV 18 Low positive 2	Pos	Pre-cytology	18	18	100% (86.9% - 100%)	4.15	4.76	5.53
		Post-cytology	18	18	100% (86.9% - 100%)	3.63	4.81	5.52
HPV 18 Moderate Positive	Pos	Pre-cytology	18	18	100% (86.9% - 100%)	4.52	5.18	5.78
		Post-cytology	18	18	100% (86.9% - 100%)	4.68	5.07	5.57

Table 80: Variance Analysis - HPV-16 Results

Signal Variability Analysis on HPV-16													
Panel ID	Aliquot	N^	Mean S/CO	Within run		Between run		Between day		Between Operator/ Instrument		Total Reproducibility	
				SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)
HPV 16 Low positive 1	Pre-cytology	4	1.50	0.06	3.77	0.00	0.00	0.00	0.00	0.70	46.73	0.70	46.88
	Post-cytology	1	1.18	—	—	—	—	—	—	—	—	—	—
HPV 16 Low positive 2	Pre-cytology	14	3.02	0.23	7.48	0.06	1.93	0.00	0.00	0.00	0.00	0.23	7.72
	Post-cytology	18	3.01	0.22	7.25	0.05	1.80	0.00	0.00	0.00	0.00	0.23	7.47
HPV 16 Moderate Positive	Pre-cytology	18	3.13	0.15	4.65	0.00	0.00	0.00	0.00	0.00	0.00	0.15	4.65
	Post-cytology	18	3.16	0.17	5.26	0.03	0.96	0.00	0.00	0.00	0.00	0.17	5.35

^Only HPV-16 positive results were analyzed for variability

Table 81: Variance Analysis - HPV-18/45 Results

Signal Variability Analysis on HPV-18/45													
Panel ID	Aliquot	N^	Mean S/CO	Within run		Between run		Between day		Between Operator/ Instrument		Total Reproducibility	
				SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)
HPV 18/45 Low positive 1	Pre-cytology	2	2.38	0.64	26.84	0.00	0.00	0.53	22.21	0.00	0.00	0.83	34.8
	Post-cytology	4	3.46	0.21	6.04	0.00	0.00	0.62	17.80	0.00	0.00	0.65	18.8
HPV 18/45 Low positive 2	Pre-cytology	18	4.73	0.35	7.46	0.00	0.00	0.23	4.90	0.20	4.18	0.47	9.9
	Post-cytology	18	4.67	0.41	8.85	0.17	3.59	0.00	0.00	0.11	2.32	0.46	9.8
HPV 18/45 Moderate Positive	Pre-cytology	18	5.15	0.28	5.37	0.00	0.00	0.17	3.33	0.00	0.00	0.33	6.3
	Post-cytology	18	5.10	0.28	5.42	0.00	0.00	0.22	4.30	0.00	0.00	0.35	6.9

^Only HPV-18/45 positive results were analyzed for variability

Cross-Reactivity

Testing with potentially cross-reactive organisms for the Aptima HPV 16 18/45 Genotype Assay was performed using the Tigris DTS System. Refer to *Cross-Reactivity* (Table 30) in the Tigris DTS System section for results.

Interference

Testing with potential interfering substances for the Aptima HPV 16 18/45 Genotype Assay was performed using the Tigris DTS System. Refer to *Interference* (Table 31) in the Tigris DTS System section for results.

Carryover for Pre- and Post-cytology Aliquots

ThinPrep 2000 System

A study was conducted to assess the carryover for post-cytology aliquot associated with the ThinPrep 2000 Processor. The following samples were evaluated for carryover:

- Post-cytology aliquot removed manually

Residual HPV-negative ThinPrep liquid Pap specimens were pooled to create multiple negative clinical pools. Aliquots were taken from each negative pool and were used to prepare the needed negative pools. Positive pools were prepared by spiking SiHa (HPV 16) cells and HeLa (HPV 18) cells at 10^4 cells/mL into negative clinical matrix. Positive and negative ThinPrep vials were alternatively processed on the ThinPrep 2000 System.

The carryover for post-cytology aliquot removed manually was 0.6% (1/160; 95% CI: 0.1%, 3.5%).

ThinPrep 5000 Processor

A study was conducted to assess the carryover for post-cytology aliquot associated with the ThinPrep 5000 Processor. The following samples were evaluated for carryover:

- Post-cytology aliquot removed manually

Residual ThinPrep liquid cytology specimens were pooled into negative pools and were aliquoted into ThinPrep Pap test vials. Positive pools were prepared by spiking HPV 16 positive (SiHa) and HPV 18 positive (HeLa) cells together to achieve a concentration of 10^4 cells/mL for each cell line and were aliquoted into ThinPrep Pap test vials. Positive and negative ThinPrep vials were alternatively processed on the ThinPrep 5000 Processor.

No carryover was observed for:

- Post-cytology aliquot removed manually: carryover was 0.0% (0/250; 95% CI: 0.0%, 1.5%)

ThinPrep Genesis Processor

A study was conducted to assess the carryover for pre-cytology and post-cytology aliquot associated with the ThinPrep Genesis Processor. The following samples were evaluated for carryover:

- Pre-cytology aliquot removed automatically by ThinPrep Genesis Processor (by "Aliquot+Slide" process on ThinPrep Genesis Processor)
- Post-cytology aliquot removed manually (after the "Aliquot+Slide" process on ThinPrep Genesis Processor)
- Post-cytology aliquot removed automatically by ThinPrep Genesis Processor (by "Aliquot" process on ThinPrep Genesis Processor after "Aliquot+Slide" process)

Positive and negative ThinPrep vials were prepared by spiking HPV expressing cells into PreservCyt solution. For each negative vial, the C33A cells (non-HPV) were spiked at 10^5 cells/mL into 20 mL PreservCyt solution as background. Each positive vial was prepared by spiking both SiHa (HPV 16) and HeLa (HPV 18) cells at 10^4 cells/mL into 20 mL PreservCyt solution containing C33A cells. Positive and negative ThinPrep vials were alternatively processed on the ThinPrep Genesis Processor.

No carryover is observed for all three types of aliquot samples:

- Pre-cytology aliquot removed automatically by ThinPrep Genesis Processor (by "Aliquot+Slide" process on ThinPrep Genesis Processor): carryover was 0.0% (0/203; 95% CI: 0.0%,1.9%)
- Post-cytology aliquot removed manually (after the "Aliquot+Slide" process on ThinPrep Genesis Processor): carryover was 0.0% (0/101; 95% CI: 0.0%, 3.7%)
- Post-cytology aliquot removed automatically by ThinPrep Genesis Processor (by "Aliquot" process on ThinPrep Genesis Processor after "Aliquot+Slide" process): carryover was 0.0% (0/102; 95% CI:0.0%, 3.6%)

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AW-34502-001 Rev. 001
2026-02