Aptima HPV Assay

For *in vitro* diagnostic use.

Rx only

Tigris® DTS® System

Panther®

General Information – Tigris DTS System and Panther System

Intended Use

The Aptima HPV assay is an *in vitro* nucleic acid amplification test for the qualitative detection of E6/E7 viral messenger RNA (mRNA) from 14 high-risk types of human papillomavirus (HPV) in cervical specimens. The high-risk HPV types detected by the assay include: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. The Aptima HPV assay does not discriminate between the 14 high-risk types. 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 assay. The assay is used with the Tigris DTS System or the Panther System.

The use of the test is indicated:

1. To screen women 21 years and older with atypical squamous cells of undetermined significance (ASC-US) cervical cytology results to determine the need for referral to colposcopy. The results of this test are not intended to prevent women from proceeding to colposcopy.
2. In women 30 years and older, the Aptima HPV assay can be used with cervical cytology to adjunctively screen to assess the presence or absence of high-risk HPV types. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.

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

WARNING:
This assay is not intended for use as a screening device for women under age 30 with normal cervical cytology.

The Aptima HPV assay is not intended to substitute for regular cervical cytology screening.

Detection of HPV using the Aptima HPV assay does not differentiate HPV types and cannot evaluate persistence of any one type.

The use of this assay 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).

The Aptima HPV assay is designed to enhance existing methods for the detection of cervical disease and should be used in conjunction with clinical information derived from other diagnostic and screening tests, physical examinations, and full medical history in accordance with appropriate patient management procedures.

Summary and Explanation of the Test

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

The HPV viral genome is a double-stranded circular DNA approximately 7900 base pairs in length. The genome has eight overlapping open reading frames. There are six early (E) genes, two late (L) genes, and one untranslated long control region. The L1 and L2 genes encode the major and minor capsid proteins. Early genes regulate HPV viral replication. The E6 and E7 genes of high-risk HPV genotypes are known oncogenes. Proteins expressed from E6/E7 polycistronic mRNA alter cellular p53 and retinoblastoma protein functions, leading to disruption of cell-cycle check points and cell genome instability.[1, 4]

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

HPV infections are very common and most women will clear HPV infections within 6 to 12 months.[2, 9] The presence of HPV nucleic acid does not mean that cervical dysplasia or cervical cancer is present. However, an effective approach for detection of cervical disease is to target those oncogenic elements of HPV that foster persistent viral infection and cellular transformation.[10]

Principles of the Procedure

The Aptima HPV assay involves three main steps, which take place in a single tube: target capture; target amplification by Transcription-Mediated Amplification (TMA®);[11] and detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA).[12] 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 assay is performed, the target mRNA is isolated from the specimen by use of capture oligomers that are linked to magnetic microparticles. The capture oligomers contain sequences complementary to specific regions of the HPV mRNA target molecules as well as a string of deoxyadenosine residues. During the hybridization step, the sequence-specific regions of the capture oligomers bind to specific regions of the HPV mRNA target molecule. The capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles, including the captured HPV mRNA target molecules bound to them, are pulled to the side of the reaction tube using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification inhibitors.

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

polymerase. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template.

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

IC is added to each reaction via the Target Capture Reagent. The IC monitors the target capture, amplification, and detection steps of the assay. IC signal in each reaction is discriminated from the HPV signal by the differential kinetics of light emission from probes with different labels.[13] IC-specific amplicon is detected using a probe with a rapid emission of light (flasher). Amplicon specific to HPV is detected using probes with relatively slower kinetics of light emission (glower). The Dual Kinetic Assay (DKA) is the method used to differentiate between the signals from the flasher and glower labels.[13]

Warnings and Precautions

A. For *in vitro* diagnostic use.

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

Laboratory Related

C. Use only supplied or specified disposable laboratory ware.

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

E. **Warning: 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.

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

Specimen Related

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

H. 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 assay should be processed using only the Aptima Specimen Transfer Kit.

I. ThinPrep liquid cytology specimens were evaluated for use with the Aptima HPV assay after processing on the ThinPrep 2000 System, the ThinPrep 5000 Processor, or the ThinPrep 5000 Processor with AutoLoader. Specimens processed using other instruments have not been evaluated.

J. 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.

K. 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.

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

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

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

Assay Related

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

P. Avoid microbial and ribonuclease contamination of reagents.

Q. Do not use kit after its expiration date.

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

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

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

U. Tips with hydrophobic plugs must be used.

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

Note: *For hazard communication information, refer to the Safety Data Sheet Library at www.hologicsds.com.*

	**Selection Reagent** ***BORIC ACID 1 – 5%*** **WARNING** H315 – Causes skin irritation H319 – Causes serious eye irritation P264 – Wash face, hands and any exposed skin thoroughly after handling P280 – Wear protective gloves/protective clothing/eye protection/face protection P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing P337 + P313 – If eye irritation persists: Get medical advice/attention P302 + P352 – IF ON SKIN: Wash with plenty of soap and water P332 + P313 – If skin irritation occurs: Get medical advice/attention P362 – Take off contaminated clothing and wash before reuse
	Target Capture Reagent ***EDTA 1 – 5%*** *Lithium Hydroxide, Monohydrate 1 – 5%* H411 – Toxic to aquatic life with long lasting effects H401 – Toxic to aquatic life

Reagent Storage and Handling Requirements

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

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

HPV Amplification Reagent

HPV Enzyme Reagent

HPV Probe Reagent

HPV Internal Control Reagent

HPV Positive Calibrators and Negative Calibrators

HPV Positive Controls and Negative Controls (Tigris DTS System only)

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

HPV Amplification Reconstitution Solution

HPV Enzyme Reconstitution Solution

HPV Probe Reconstitution Solution

HPV Target Capture Reagent

HPV Selection Reagent

Wash Solution

Oil Reagent

Buffer for Deactivation Fluid

Auto Detect Reagent 1

Auto Detect Reagent 2

Aptima System Fluid Preservative (Tigris DTS System only)

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

   HPV Amplification Reagent

   HPV Enzyme Reagent

   HPV Probe Reagent

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

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

F. The Aptima HPV assay reagents are stable for a cumulative of 48 hours when stored on-board the Tigris DTS System.

G. The Aptima HPV assay reagents are stable for a cumulative of 72 hours when stored on-board the Panther System.

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

I. Do not freeze reagents.

Specimen Collection and Storage

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. Prior to or after cytology processing with the ThinPrep 2000 System, the ThinPrep 5000 Processor, or the ThinPrep 5000 Processor with AutoLoader, transfer 1 mL of the ThinPrep liquid 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 specimens at 2°C to 30°C.
   2. Specimens should be transferred to an Aptima Specimen Transfer tube within 105 days of collection.
   3. Prior to transfer, ThinPrep liquid cytology specimens should be stored at 2°C to 30°C, with no more than 30 days at temperatures above 8°C.
   4. ThinPrep liquid cytology specimens transferred to an Aptima Specimen Transfer tube may be stored at 2°C to 30°C for up to 60 days.
   5. If longer storage is needed, the ThinPrep liquid cytology specimen or the ThinPrep liquid cytology specimen diluted into the Aptima Specimen Transfer tube may be stored at -20°C to -70°C for up to 24 months.

C. Specimen storage after testing

   1. Specimens that have been assayed must be stored upright in a rack.
   2. Specimen tubes should be covered with a new, clean plastic or foil barrier.

3. If assayed specimens need to be frozen or shipped, remove penetrable caps 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 transportation regulations.*

Tigris DTS System

Reagents and Materials Provided

Aptima HPV Assay Kit, 250 tests, Cat. No. 303012 (4 boxes)

Calibrators and Controls may be purchased separately. See individual catalog numbers below

Note:

Aptima HPV Assay Refrigerated Box
(store at 2°C to 8°C upon receipt)

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

Aptima HPV Assay Room Temperature Box
(store at 15°C to 30°C upon receipt)

Symbol	Component	Quantity
AR	**HPV Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 vial
ER	**HPV Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 vial
PR	**HPV Probe Reconstitution Solution** *Succinate buffered solution containing < 5% detergent.*	1 vial
S	**HPV Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 vial
TCR	**HPV 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 Assay Calibrators Box (Cat. No. 303010)
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
PCAL	**HPV Positive Calibrator** *Non-infectious HPV 16 in vitro transcript at 1000 copies per mL in a buffered solution containing < 5% detergent.*	5 vials
NCAL	**HPV Negative Calibrator** *Buffered solution containing < 5% detergent.*	5 vials

Aptima HPV Assay Controls Box (Cat. No. 303011)
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
PC	**HPV Positive Control** *Lysed, inactivated HPV Negative and HPV Positive cultured cells at 25 cells per mL in a buffered solution containing < 5% detergent.*	5 vials
NC	**HPV Negative Control** *Lysed, inactivated HPV Negative cultured cells in a buffered solution containing < 5% detergent.*	5 vials

Materials Required But Available Separately

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

	Cat. No.
Tigris DTS System	105118
Tigris DTS System Run Kit	301191
Multi-tube Units (MTU)	*104772-02*
MTU/Tiplet Waste Bag	*900907*
MTU Waste Deflectors	*900931*
MTU Waste Covers	*105523*
Aptima 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 conductive, liquid sensing	10612513 (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 Amplification and Probe Reagent reconstitution solutions	CL0041
Spare Caps for Enzyme Reagent reconstitution solution	501616
Spare Caps for TCR and Selection Reagent	CL0040
Bleach, minimum 5% or 0.7 M sodium hypochlorite solution	—
Water for the Tigris DTS System *consult the Tigris DTS System Operator's Manual for specifications*	—
Disposable gloves	—

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 Preparation of a New Kit

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

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

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

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

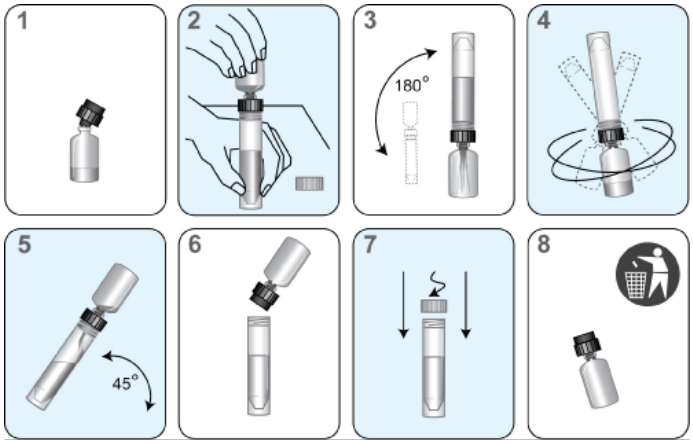

Figure 1. Tigris DTS System reconstitution process

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

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

C. Reagent Preparation for Previously Reconstituted Reagents

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

D. Sample Handling

1. Allow the samples (calibrators, controls (including Aptima HPV Controls and any user provided external quality control samples), and specimens) to reach room temperature prior to processing.
2. **Do not vortex samples**.
3. Inspect sample tubes before loading into the racks. If a sample tube contains bubbles or has a lower volume than is typically observed, centrifuge the tube for 5 minutes at 420 RCF to ensure that 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.

Procedural Notes

A. Calibrators

1. Each worklist must contain 3 replicates of the Negative Calibrator and Positive Calibrator. In order to work properly with the Aptima HPV assay Software, the Negative Calibrator must be in the first tube position of the first rack of the worklist and the Positive Calibrator must be in the second tube position of the first rack of the worklist.
2. Attempts to pipette more than three replicates from a calibrator tube can lead to insufficient volume errors.

B. Controls

1. The Aptima HPV assay software requires beginning and end run controls. The Negative Control must be in the third tube position of the first rack and the second to last tube position of the last rack of the worklist. The Positive Control must be in the fourth tube position of the first rack and the last tube position of the last rack of the worklist.
2. Attempts to pipette more than once from a control tube can lead to insufficient volume errors.

C. Temperature

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

D. 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 Assay, 250 tests, Cat. No. 303585 (3 boxes)
Aptima HPV Assay, 100 tests, Cat. No. 303570 (3 boxes)

Calibrators may be purchased separately. See the individual catalog numbers below.

Aptima HPV Assay Refrigerated Box
(store at 2°C to 8°C upon receipt)

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

Aptima HPV Assay Room Temperature Box
(store at room temperature, 15°C to 30°C upon receipt)

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

Aptima HPV Assay Calibrators Box (Cat. No. 303010)
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
PCAL	**HPV Positive Calibrator** *Non-infectious HPV 16 in vitro transcript at 1000 copies per mL in a buffered solution containing < 5% detergent.*	5 vials
NCAL	**HPV Negative Calibrator** *Buffered solution containing < 5% detergent.*	5 vials

Materials Required But Available Separately

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

	Cat. No.
Panther System	303095
Panther Run Kit	303096
Aptima Assay Fluids Kit	*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 conductive, liquid sensing	10612513 (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 250 test kits:	
Amplification Reagent and Probe Reagent reconstitution solutions	*CL0041*
Enzyme Reagent reconstitution solution	*501616*
TCR and Selection Reagent	*CL0040*
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% or 0.7 M sodium hypochlorite solution	—
Disposable gloves	—

Panther System Test Procedure

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

A. Work Area Preparation

Clean work surfaces where reagents and samples will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35 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 water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface on which the reagents and samples will be prepared with clean, plastic-backed absorbent laboratory bench covers.

B. Reagent Preparation of a New Kit

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

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

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

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

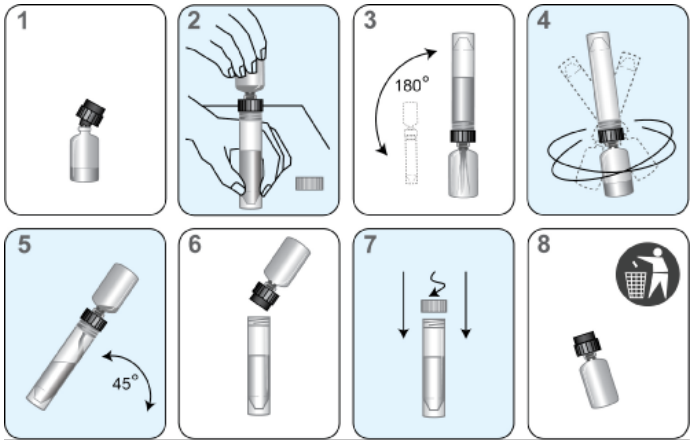

Figure 2. Panther System reconstitution process

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

D. Sample Handling

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

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

E. System Preparation

Set up the system according to the instructions in the *Panther System Operator's Manual* and the *Procedural Notes* section below. Make sure that the appropriately sized reagent racks and TCR adapters are used.

Procedural Notes

A. Calibrators

1. To work properly with the Aptima HPV assay Software on the Panther System, three replicates of the Positive Calibrator and three replicates of the Negative Calibrator are required. One vial of each calibrator may be loaded in any rack position in any Sample Bay Lane on the Panther System. Specimen pipetting will begin when one of the following two conditions has been met:
   a. A Positive and Negative Calibrator are currently being processed by the system.
   b. Valid results for the calibrators are registered on the 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 system.
   c. The associated assay reagent kit has exceeded the stability limits.

3. Attempts to pipette more than three 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 replicate.
- An invalid Negative Control (Tigris DTS System only).
- An invalid Positive Control (Tigris DTS System only).

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.

Negative Calibrator	
Analyte	*≥ 0 and ≤ 45,000 RLU*
IC	*≥ 75,000 and ≤ 400,000 RLU*
Positive Calibrator	
Analyte	*≥ 480,000 and ≤ 1,850,000 RLU*
IC	*≤ 450,000 RLU*

C. IC Cutoff

The IC cutoff is determined from the IC (flasher) signal from the valid Negative Calibrator replicates.

$$\textbf{IC Cutoff} = 0.5 \times [\text{mean IC RLU of the valid Negative Calibrator replicates}]$$

D. Analyte Cutoff

The analyte cutoff is determined from the analyte (glower) signal from the valid Negative Calibrator replicates as well as the analyte signal from the valid Positive Calibrator replicates.

$$\textbf{Analyte Cutoff} = [\text{mean analyte RLU of the valid Negative Calibrator replicates}] + [0.09 \times \text{mean analyte RLU of the valid Positive Calibrator replicates}]$$

E. Analyte Signal to Cutoff (S/CO)

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

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

F. Control Acceptance Criteria (Tigris DTS System only)

The Negative Control must have a valid negative result (IC RLU ≥ IC cutoff and analyte S/CO < 0.50). The Positive Control must have a valid positive result (analyte S/CO ≥ 0.50).

Test Interpretation – Tigris DTS System and Panther System

Test results are automatically determined by the assay software. A test result may be negative, positive, or invalid as determined by the IC RLU and the S/CO for the Analyte. A test result may also be invalid due to other parameters (abnormal kinetic curve shape) being outside the normal expected ranges. Invalid test results should be repeated.

Aptima HPV Assay Result	Criteria
Negative	*Analyte S/CO < 0.50* *IC≥ IC Cutoff* *IC≤ 2,000,000 RLU*
Positive	*Analyte S/CO ≥ 0.50* *IC≤ 2,000,000 RLU* *Analyte ≤ 13,000,000 RLU*
Invalid	*Analyte S/CO < 0.50 and IC< IC Cutoff* *or* *IC> 2,000,000 RLU* *or* *Analyte > 13,000,000 RLU*

Note: *Negative results are not intended to prevent women from proceeding to colposcopy.*

Note: *Negative results indicate HPV E6/E7 mRNA was not detected.*

Note: *Negative results may occur with HPV E6/E7 mRNA concentrations that are below the pre-set threshold.*

Note: *Positive results indicate the presence of HPV E6/E7 mRNA of any one or more of the high-risk types.*

Note: *Results of this test should only be interpreted in conjunction with information available from clinical evaluation of the patient and patient history.*

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 assay has not been evaluated for HPV vaccinated individuals.

B. The Aptima HPV 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 assay.

E. Aptima HPV 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, or the ThinPrep 5000 Processor with AutoLoader.

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

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

H. A negative Aptima HPV assay result does not exclude the possibility of cytologic abnormalities or of future or underlying CIN2, CIN3, or cancer.

I. Personal lubricants that contain Polyquaternium 15 may interfere with the performance of the assay when present at concentrations greater than 0.025% (v/v or w/v) of a test sample.

J. Anti-fungal medications that contain tioconazole may interfere with the performance of the assay when present at concentrations greater than 0.075% (w/v) of a test sample.

K. The Aptima HPV 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.

L. The Aptima HPV assay detects E6/E7 viral messenger RNA (mRNA) of the high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. This test does not detect E6/E7 mRNA of HPV low-risk types (e.g. 6, 11, 42, 43, 44) since there is no clinical utility for testing of low-risk HPV types for cervical cancer screening purposes.[14]

M. Detection of high-risk HPV 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 HSIL or underlying high-grade CIN, nor does it imply that CIN2, CIN3, or cancer will develop. Most women infected with one or more high-risk HPV types do not develop CIN2, CIN3, or cancer.

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

P. Use of this device must be limited to personnel trained in the use of the Aptima HPV assay.

Q. Cross-contamination of samples can cause false positive results. The carry-over rate of the Aptima HPV assay on the Tigris DTS System and the Panther System was 0.7% and 0.4% respectively, as determined in non-clinical studies.

R. Cross-contamination of specimens can cause false positive results. The carry-over rate of specimens processed on the ThinPrep 5000 Processor prior to testing with the Aptima HPV assay on the Panther System was determined to be 0.4%.

S. The Aptima HPV assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

T. False positive results may occur with this test. *In vitro* transcripts from low-risk HPV genotypes 26, 67, 70, and 82 exhibited cross-reactivity with the Aptima HPV assay.

U. The positive control material (Tigris DTS System only) is not intended to monitor performance at the assay cutoff.

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, for which age is the greatest contributor.[15,16] Many studies have investigated HPV prevalence as determined by the detection of HPV DNA, however few studies report prevalence based on detection of HPV oncogenic mRNA. Women from a variety of clinical sites (n=18) representing a wide geographic distribution and a diverse population (10 states within the United States) were enrolled in a prospective clinical study known as the CLEAR trial.[17] The prevalence of HPV mRNA-positive samples observed in the clinical study 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 by Age Group, Testing Site, and All Combined

	Positivity Rate % (x/n)	
	ASC-US Population (≥ 21 Years)	**NILM Population (≥ 30 Years)**
All	41.8 (400/958)	5.0 (540/10,871)
Age Group (years)		
21 to 29	60.3 (252/418)	N/A
30 to 39	36.8 (98/266)	6.9 (289/4199)
≥ 40	18.2 (50/274)	3.8 (251/6672)
Testing Site		
1	41.6 (134/322)	4.7 (172/3682)
2	41.4 (150/362)	5.2 (194/3702)
3	42.3 (116/274)	5.0 (174/3487)

N/A = Not Applicable

Aptima HPV Assay on the Tigris DTS System Clinical Study Design

A prospective, multicenter US clinical study known as 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).[17] The CLEAR trial included a baseline evaluation and a 3 year follow-up evaluation.

CLEAR Trial – Baseline Evaluation

At baseline of the CLEAR trial (Baseline Phase), women were enrolled into either the ASC-US Study or the NILM Study based on 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. The NILM Study was designed to support the adjunctive screening claim for women 30 years and older, since women in this age range with cytology results greater than ASC-US should proceed to colposcopy regardless of their HPV status.[14]

Women from 18 clinical sites, primarily obstetrics/gynecology clinics, which covered a wide geographic distribution and a diverse population, were enrolled. Eligible women were assigned to the ASC-US Study or NILM Study based on their referral ThinPrep liquid based cytology specimen. At baseline, residual referral 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.

At baseline, all women in the ASC-US Study were referred to colposcopy, regardless of their HPV 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. The randomly selected women who were negative for both assays were included to correct for verification bias with adjusted performance estimates generated using a multiple imputation method. An ECC biopsy was obtained from each woman who attended colposcopy. Punch biopsies were obtained from visible lesions only (directed 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 woman'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 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. Clinical performance of the FDA-approved HPV DNA test was also determined for direct comparison to the Aptima HPV assay results.

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 colposcocpy visit at the 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 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 NILM study 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 results with the cumulative 3-year risk of cervical disease in women with baseline negative Aptima HPV 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 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 Assay Clinical Performance

In total, there were 1252 women 21 years of age and older with ASC-US cytology results enrolled in the ASC-US Study. Of these, 294 women were withdrawn and 19 had an undetermined disease diagnosis; all were excluded from analysis. The remaining 939 evaluable women were 21 years of age and older with ASC-US cytology results, Aptima HPV assay results, and conclusive disease status. Ninety-one (91) women had ≥CIN2 and forty-one (41) had ≥CIN3. Prevalence of ≥CIN2 and ≥CIN3 in evaluable women with ASC-US cytology results were 9.7% and 4.4%, respectively. The results of the Aptima HPV assay by the Consensus Histology Review Panel diagnoses are presented in Table 2.

Table 2: ASC-US ≥ 21 Years Population: Results of the Aptima HPV Assay by Consensus Histology Review Panel Diagnosis

Aptima HPV Assay Result*	HPV DNA Test	Consensus Histology Review Panel Diagnosis						
		Undetermined**	Normal	CIN1	CIN2	CIN3	Cancer	Total
Positive	Positive	6	170	113	41	32	1	363
Positive	Negative	0	7	0	1	2	0	10
Positive	No Result***	0	14	11	0	2	0	27
Negative	Positive	0	47	13	2	3	0	65
Negative	Negative	10	371	55	6	1	0	443
Negative	No Result***	3	40	7	0	0	0	50
Total		19	649	199	50	40	1****	958

*All samples had final valid results (upon initial testing or after resolution of initial invalids per procedure).
**19 subjects 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).
***77 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.
****One subject had adenocarcinoma in situ (AIS).

Clinical performance estimates of the Aptima HPV assay including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the detection of ≥CIN2 and ≥CIN3 based on evaluating all biopsies and including only directed biopsies are shown in Table 3, as are the estimates for the FDA-approved HPV DNA test.

Table 3: ASC-US ≥ 21 Years Population: Performance of the Aptima HPV Assay and an FDA-approved HPV DNA Test for Detection of ≥CIN2 and ≥CIN3

		Aptima HPV Assay N=939		HPV DNA Test N=865*	
	Performance	**Estimate**	**(95% CI)**	**Estimate**	**(95% CI)**
≥CIN2	**All Biopsies**				
	Sensitivity (%)	86.8 (79/91)	(78.4, 92.3)	88.8 (79/89)	(80.5, 93.8)
	Specificity (%)	62.9 (533/848)	(59.6, 66.0)	55.8 (433/776)	(52.3, 59.3)
	PPV (%)	20.1 (79/394)	(18.1, 22.0)	18.7 (79/422)	(17.0, 20.4)
	NPV (%)	97.8 (533/545)	(96.5, 98.8)	97.7 (433/443)	(96.2, 98.8)
	Prevalence (%)	9.7 (91/939)		10.3 (89/865)	
	Directed Biopsies**				
	Sensitivity (%)	93.3 (56/60)	(84.1, 97.4)	93.2 (55/59)	(83.8, 97.3)
	Specificity (%)	61.5 (539/876)	(58.3, 64.7)	54.5 (438/804)	(51.0, 57.9)
	PPV (%)	14.2 (56/393)	(12.7, 15.6)	13.1 (55/421)	(11.7, 14.2)
	NPV (%)	99.3 (539/543)	(98.3, 99.8)	99.1 (438/442)	(97.9, 99.7)
	Prevalence (%)	6.4 (60/936)		6.8 (59/863)	
≥CIN3	**All Biopsies**				
	Sensitivity (%)	90.2 (37/41)	(77.5, 96.1)	92.3 (36/39)	(79.7, 97.3)
	Specificity (%)	60.2 (541/898)	(57.0, 63.4)	53.3 (440/826)	(49.9, 56.6)
	PPV (%)	9.4 (37/394)	(8.1, 10.4)	8.5 (36/422)	(7.4, 9.4)
	NPV (%)	99.3 (541/545)	(98.3, 99.8)	99.3 (440/443)	(98.3, 99.8)
	Prevalence (%)	4.4 (41/939)		4.5 (39/865)	
	Directed Biopsies**				
	Sensitivity (%)	93.1 (27/29)	(78.0, 98.1)	96.4 (27/28)	(82.3, 99.4)
	Specificity (%)	59.6 (541/908)	(56.4, 62.7)	52.8 (441/836)	(49.4, 56.1)
	PPV (%)	6.9 (27/394)	(5.8, 7.6)	6.4 (27/422)	(5.5, 7.0)
	NPV (%)	99.6 (541/543)	(98.8, 100)	99.8 (441/442)	(98.9, 100)
	Prevalence (%)	3.1 (29/937)		3.2 (28/864)	

*74 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.
**Consensus histology result was derived using only results from directed biopsies. Women with no directed biopsies reflect a normal colposcopy and are included in these analyses as non-diseased (<CIN2 or <CIN3, as appropriate). A consensus was not always reached when only directed biopsies were included.

When evaluating all biopsies, clinical sensitivity estimates of the Aptima HPV assay and the FDA-approved HPV DNA test for the detection of ≥CIN2 and ≥CIN3, where both assay results are available, were similar (differences in sensitivity estimates were not statistically significant). For ≥CIN2 the sensitivity difference was -2.3% (95% CI: -9.9%, 4.8%). Clinical specificity estimates of the Aptima HPV assay for the detection of ≥CIN2 and ≥CIN3 were higher than those of the FDA-approved HPV DNA test (differences in specificity estimates were statistically significant). For ≥CIN2, the specificity difference was 6.8% (95% CI: 4.9%, 9.0%). NPVs were similar but for the detection of ≥CIN2, the PPV for the Aptima HPV assay was slightly higher than PPV for the FDA-approved HPV DNA test (20.1% vs 18.7%).

Of the 91 ≥CIN2 cases, 60 (65.9%) were identified in directed biopsies and 31 (34.1%) were identified from random and/or ECC biopsies (i.e., not in directed biopsies). These findings are comparable to results from published studies, in which approximately 25% to 40% of ≥CIN2 cases were identified from random and/or ECC biopsy specimens only.[18,19] Using only directed biopsies to determine disease status (assuming women with no directed biopsies had normal histology results because no visible lesions were present), prevalence of ≥CIN2 and ≥CIN3 in the study were 6.4% and 3.1%, respectively. The clinical sensitivity estimates for the detection of ≥CIN2 and ≥CIN3 were higher for both tests using directed biopsies only than estimates calculated using all biopsies. For both assays, clinical specificity using only directed biopsies was similar to the specificity obtained with all biopsies included. Accordingly, when using only directed biopsies, the Aptima HPV assay specificity was significantly higher than that of the FDA-approved HPV DNA test.

Clinical performance estimates of the Aptima HPV assay and the FDA-approved HPV DNA test are shown by age group in Table 4 and Table 5 (≥CIN2 and ≥CIN3, respectively, based on evaluating all biopsies).

Table 4: ASC-US ≥ 21 Years Population: Performance of the Aptima HPV Assay and an FDA-approved HPV DNA Test for Detection of ≥CIN2 by Age Group

		Aptima HPV Assay N=939		HPV DNA Test N=865*	
	Performance	**Estimate**	**(95% CI)**	**Estimate**	**(95% CI)**
21 to 29 Years		N=415		N=389	
	Sensitivity (%)	90.2 (55/61)	(80.2, 95.4)	94.9 (56/59)	(86.1, 98.3)
	Specificity (%)	44.9 (159/354)	(39.8, 50.1)	35.5 (117/330)	(30.5, 40.8)
	PPV (%)	22.0 (55/250)	(19.6, 24.2)	20.8 (56/269)	(19.0, 22.5)
	NPV (%)	96.4 (159/165)	(93.0, 98.5)	97.5 (117/120)	(93.6, 99.4)
	Prevalence (%)	14.7 (61/415)		15.2 (59/389)	
30 to 39 Years		N=262		N=239	
	Sensitivity (%)	90.0 (18/20)	(69.9, 97.2)	80.0 (16/20)	(58.4, 91.9)
	Specificity (%)	68.2 (165/242)	(62.1, 73.7)	61.6 (135/219)	(55.1, 67.8)
	PPV (%)	18.9 (18/95)	(14.7, 22.7)	16.0 (16/100)	(11.8, 19.6)
	NPV (%)	98.8 (165/167)	(96.5, 99.8)	97.1 (135/139)	(94.1, 99.1)
	Prevalence (%)	7.6 (20/262)		8.4 (20/239)	
≥ 40 Years		N=262		N=237	
	Sensitivity (%)	60.0 (6/10)	(31.3, 83.2)	70.0 (7/10)	(39.7, 89.2)
	Specificity (%)	82.9 (209/252)	(77.8, 87.1)	79.7 (181/227)	(74.0, 84.4)
	PPV (%)	12.2 (6/49)	(5.8, 18.4)	13.2 (7/53)	(6.9, 18.7)
	NPV (%)	98.1 (209/213)	(96.6, 99.4)	98.4 (181/184)	(96.6, 99.6)
	Prevalence (%)	3.8 (10/262)		4.2 (10/237)	

*74 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

Table 5: ASC-US ≥ 21 Years Population: Performance of the Aptima HPV Assay and an FDA-approved HPV DNA Test for Detection of ≥CIN3 by Age Group

		Aptima HPV Assay N=939		HPV DNA Test N=865*	
	Performance	**Estimate**	**(95% CI)**	**Estimate**	**(95% CI)**
21 to 29 Years		N=415		N=389	
	Sensitivity (%)	96.3 (26/27)	(81.7, 99.3)	100 (25/25)	(86.7, 100)
	Specificity (%)	42.3 (164/388)	(37.5, 47.2)	33.0 (120/364)	(28.3, 38.0)
	PPV (%)	10.4 (26/250)	(8.9, 11.4)	9.3 (25/269)	(8.2, 10.0)
	NPV (%)	99.4 (164/165)	(97.2, 100)	100 (120/120)	(97.5, 100)
	Prevalence (%)	6.5 (27/415)		6.4 (25/389)	
30 to 39 Years		N=262		N=239	
	Sensitivity (%)	88.9 (8/9)	(56.5, 98.0)	77.8 (7/9)	(45.3, 93.7)
	Specificity (%)	65.6 (166/253)	(59.6, 71.2)	59.6 (137/230)	(53.1, 65.7)
	PPV (%)	8.4 (8/95)	(5.2, 10.4)	7.0 (7/100)	(3.9, 9.1)
	NPV (%)	99.4 (166/167)	(97.6, 100)	98.6 (137/139)	(96.4, 99.8)
	Prevalence (%)	3.4 (9/262)		3.8 (9/239)	
≥ 40 Years		N=262		N=237	
	Sensitivity (%)	60.0 (3/5)	(23.1, 88.2)	80.0 (4/5)	(37.6, 96.4)
	Specificity (%)	82.1 (211/257)	(77.0, 86.3)	78.9 (183/232)	(73.2, 83.6)
	PPV (%)	6.1 (3/49)	(1.6, 10.2)	7.5 (4/53)	(2.9, 10.7)
	NPV (%)	99.1 (211/213)	(98.0, 99.9)	99.5 (183/184)	(98.2, 100)
	Prevalence (%)	1.9 (5/262)		2.1 (5/237)	

*74 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

The absolute risk of disease (≥CIN2 and ≥CIN3, based on evaluating all biopsies) by Aptima HPV assay result and the relative risk of disease for positive versus negative Aptima HPV assay results are shown in Table 6, as are the estimates for the FDA-approved HPV DNA test. The relative risk of ≥CIN2 was 9.1 (95% CI: 5.0, 16.5), indicating that a woman who was Aptima HPV assay positive was 9.1 times as likely to have ≥CIN2 than a woman who was Aptima HPV assay negative. The relative risk of ≥CIN3 was 12.8 (95% CI: 4.6, 35.6).

Table 6: ASC-US ≥ 21 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an FDA-approved HPV DNA Test

		Aptima HPV Assay N=939		HPV DNA Test N=865*	
	Assay Result	Absolute Risk (95% CI)	Relative Risk (95% CI)	Absolute Risk (95% CI)	Relative Risk (95% CI)
≥CIN2	Positive	20.1 (79/394) (18.1, 22.0)	9.1 (5.0, 16.5)	18.7 (79/422) (17.0, 20.4)	8.3 (4.4, 15.8)
	Negative	2.2 (12/545) (1.2, 3.5)		2.3 (10/443) (1.2, 3.8)	
	Prevalence (%)	9.7 (91/939)		10.3 (89/865)	
≥CIN3	Positive	9.4 (37/394) (8.1, 10.4)	12.8 (4.6, 35.6)	8.5 (36/422) (7.4, 9.4)	12.6 (3.9, 40.6)
	Negative	0.7 (4/545) (0.2, 1.7)		0.7 (3/443) (0.2, 1.7)	
	Prevalence (%)	4.4 (41/939)		4.5 (39/865)	

*74 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

Absolute and relative risk estimates of disease (≥CIN2 and ≥CIN3, based on evaluating all biopsies) for the Aptima HPV assay and the FDA-approved HPV DNA test are shown by age group in Table 7.

Table 7: ASC-US ≥ 21 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an FDA-approved HPV DNA Test by Age Group

	Age	Assay Result	Aptima HPV Assay N=939		HPV DNA Test N=865*	
			Absolute Risk (95% CI)	Relative Risk (95% CI)	Absolute Risk (95% CI)	Relative Risk (95% CI)
≥CIN2	21 to 29 Years		N=415		N=389	
		Positive	22.0 (55/250) (19.6, 24.2)	6.1 (2.7, 13.7)	20.8 (56/269) (19.0, 22.5)	8.3 (2.7, 26.1)
		Negative	3.6 (6/165) (1.5, 7.0)		2.5 (3/120) (0.6, 6.4)	
		Prevalence (%)	14.7 (61/415)		15.2 (59/389)	
	30 to 39 Years		N=262		N=239	
		Positive	18.9 (18/95) (14.7, 22.7)	15.8 (3.8, 66.7)	16.0 (16/100) (11.8, 19.6)	5.6 (1.9, 16.1)
		Negative	1.2 (2/167) (0.2, 3.5)		2.9 (4/139) (0.9, 5.9)	
		Prevalence (%)	7.6 (20/262)		8.4 (20/239)	
	≥ 40 Years		N=262		N=237	
		Positive	12.2 (6/49) (5.8, 18.4)	6.5 (1.9, 22.2)	13.2 (7/53) (6.9, 18.7)	8.1 (2.2, 30.2)
		Negative	1.9 (4/213) (0.6, 3.4)		1.6 (3/184) (0.4, 3.4)	
		Prevalence (%)	3.8 (10/262)		4.2 (10/237)	
≥CIN3	21 to 29 Years		N=415		N=389	
		Positive	10.4 (26/250) (8.9, 11.4)	17.2 (2.4, 125)	9.3 (25/269) (8.2, 10.0)	Not Calculable
		Negative	0.6 (1/165) (0.0, 2.8)		0.0 (0/120) (0.0, 2.5)	
		Prevalence (%)	6.5 (27/415)		6.4 (25/389)	
	30 to 39 Years		N=262		N=239	
		Positive	8.4 (8/95) (5.2, 10.4)	14.1 (1.8, 111)	7.0 (7/100) (3.9, 9.1)	4.9 (1.0, 22.9)
		Negative	0.6 (1/167) (0.0, 2.4)		1.4 (2/139) (0.2, 3.6)	
		Prevalence (%)	3.4 (9/262)		3.8 (9/239)	
	≥ 40 Years		N=262		N=237	
		Positive	6.1 (3/49) (1.6, 10.2)	6.5 (1.1, 38.0)	7.5 (4/53) (2.9, 10.7)	13.9 (1.6, 122)
		Negative	0.9 (2/213) (0.1, 2.0)		0.5 (1/184) (0.0, 1.8)	
		Prevalence (%)	1.9 (5/262)		2.1 (5/237)	

*74 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

NILM ≥ 30 Years Population: Aptima HPV Assay Clinical Performance at Baseline

In total, there were 11,644 women with NILM cytology results enrolled in the NILM Study. Of these, 773 women were withdrawn and excluded from the baseline evaluation. The remaining 10,871 evaluable women were 30 years of age and older with NILM cytology results and Aptima HPV assay results. Of the 540 women with positive Aptima HPV assay results, 335 attended colposcopy at baseline. Of the 10,331 women with negative Aptima HPV assay results, 530 attended colposcopy at baseline. Twenty (20) women had ≥CIN2 and eleven (11) had ≥CIN3; 799 women had Normal/CIN1 histology; 46 women had undetermined disease status. The results of the Aptima HPV assay by the Consensus Histology Review Panel diagnosis at baseline are presented in Table 8.

Table 8: NILM ≥ 30 Years Population: Results of the Aptima HPV Assay and an FDA-approved HPV DNA Test by Consensus Histology Review Panel Diagnosis at Baseline

Aptima HPV Assay Result*	HPV DNA Test	Consensus Histology Review Panel Diagnosis						
		Undetermined	Normal	CIN1	CIN2	CIN3	Cancer	Total
Positive	Positive	11	212	11	4	7	2	247
Positive	Negative	7	59	0	1	0	1	68
Positive	No Result**	3	16	1	0	0	0	20
Negative	Positive	10	170	8	2	1	0	191
Negative	Negative	15	313	9	1	0	0	338
Negative	No Result**	0	0	0	1	0	0	1
Total		46	770	29	9	8	3***	865

*All samples had final valid results (upon initial testing or after resolution of initial invalids per procedure).
**21 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.
***Three women had adenocarcinoma in situ (AIS).

In total, 10,052 women had unverified (including undetermined) disease status at baseline (Table 9). Because only randomly selected women with negative results for both the Aptima HPV assay and the FDA-approved HPV DNA test were referred to colposcopy, the proportion of women with unverified disease status was high in this group (96.6%). To adjust for this verification bias, a multiple imputation method was used to estimate the number of women with disease that would have been identified if all women had undergone colposcopy. Both verification-bias adjusted performance estimates and unadjusted performance estimates based on the 819 women with verified disease status at baseline are presented.

Table 9: NILM ≥ 30 Years Population: Classification of Evaluable NILM Women by Aptima HPV Assay and an FDA-approved HPV DNA Test Results, Disease Status (≥CIN2 and ≥CIN3), and Disease Verification Status at Baseline

Aptima HPV 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	360	13	223	9	227	124 (34.4%)
Positive	Negative	150	2	59	1	60	89 (59.3%)
Positive	No Result**	30	0	17	0	17	13 (43.3%)
Negative	Positive	306	3	178	1	180	125 (40.8%)
Negative	Negative	9420	1	322	0	323	9097 (96.6%)
Negative	No Result**	605	1	0	0	1	604 (99.8%)
Total		10,871	20	799	11	808	10,052 (92.5%)

*All samples had final results (upon initial testing or after resolution of initial invalids per procedure).
**635 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

The adjusted prevalence of ≥CIN2 and ≥CIN3 in women with NILM cytology results were 0.9% and 0.4%, respectively. The adjusted absolute and relative risk estimates for detection of ≥CIN2 and ≥CIN3 at baseline are shown in Table 10. The adjusted relative risk of ≥CIN2 was 8.1 (95% CI: 2.3, 28.1), indicating that a woman who was Aptima HPV assay positive is 8.1 times as likely to have ≥CIN2 than a woman who is Aptima HPV assay negative. The adjusted relative risk of ≥CIN3 was 34.5 (95% CI: 2.7, 443.3). The unadjusted absolute and relative risk estimates for detection of ≥CIN2 and ≥CIN3 at baseline are shown overall in Table 11 and by age group in Table 12.

Table 10: NILM ≥ 30 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an FDA-approved HPV DNA Test (Verification-Bias Adjusted Estimates) at Baseline

		Aptima HPV Assay		HPV DNA Test	
	Assay Result	**Absolute Risk (95% CI)**	**Relative Risk (95% CI)**	**Absolute Risk (95% CI)**	**Relative Risk (95% CI)**
≥CIN2	**Positive**	4.7 (2.9, 7.6)	8.1 (2.3, 28.1)	3.7 (2.3, 6.0)	7.3 (1.6, 33.4)
	Negative	0.6 (0.2, 1.9)		0.5 (0.1, 2.1)	
	Prevalence (%)	0.9		0.9	
≥CIN3	**Positive**	3.3 (1.4, 7.6)	34.5 (2.7, 443.3)	2.3 (1.3, 4.1)	21.0 (1.0, 423.4)
	Negative	0.1 (0.0, 1.6)		0.1 (0.0, 2.4)	
	Prevalence (%)	0.4		0.4	

Table 11: NILM ≥ 30 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an FDA-approved HPV DNA Test (Unadjusted Estimates) at Baseline

		Aptima HPV Assay N=819		HPV DNA Test N=801*	
	Assay Result	**Absolute Risk (95% CI)**	**Relative Risk (95% CI)**	**Absolute Risk (95% CI)**	**Relative Risk (95% CI)**
≥CIN2	**Positive**	4.8 (15/314) (3.4, 5.8)	4.8 (1.8, 13.1)	3.8 (16/417) (2.9, 4.4)	4.9 (1.4, 16.7)
	Negative	1.0 (5/505) (0.4, 1.9)		0.8 (3/384) (0.2, 1.9)	
	Prevalence (%)	2.4 (20/819)		2.4 (19/801)	
≥CIN3	**Positive**	3.2 (10/314) (2.2, 3.7)	16.1 (2.1, 125)	2.4 (10/417) (1.6, 2.7)	9.2 (1.2, 71.6)
	Negative	0.2 (1/505) (0.0, 0.9)		0.3 (1/384) (0.0, 1.1)	
	Prevalence (%)	1.3 (11/819)		1.4 (11/801)	

*18 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

Table 12: NILM ≥ 30 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an FDA-approved HPV DNA Test by Age Group (Unadjusted Estimates) at Baseline

	Age	Assay Result	Aptima HPV Assay N=819		HPV DNA Test N=801*	
			Absolute Risk (95% CI)	Relative Risk (95% CI)	Absolute Risk (95% CI)	Relative Risk (95% CI)
≥CIN2	30 to 39 Years		N=384		N=377	
		Positive	4.8 (8/167) (2.1, 9.2)	10.4 (1.3, 82.3)	3.2 (7/216) (1.3, 6.6)	2.6 (0.5, 12.4)
		Negative	0.5 (1/217) (0.0, 2.5)		1.2 (2/161) (0.2, 4.4)	
		Prevalence (%)	2.3 (9/384)		2.4 (9/377)	
	≥ 40 Years		N=435		N=424	
		Positive	4.8 (7/147) (1.9, 9.6)	3.4 (1.0, 11.5)	4.5 (9/201) (2.1, 8.3)	10.0 (1.3, 78.1)
		Negative	1.4 (4/288) (0.4, 3.5)		0.4 (1/223) (0.0, 2.5)	
		Prevalence (%)	2.5 (11/435)		2.4 (10/424)	
≥CIN3	30 to 39 Years		N=384		N=377	
		Positive	3.0 (5/167) (1.0, 6.8)	6.5 (0.8, 55.1)	2.3 (5/216) (0.8, 5.3)	3.7 (0.4, 31.6)
		Negative	0.5 (1/217) (0.0, 2.5)		0.6 (1/161) (0.0, 3.4)	
		Prevalence (%)	1.6 (6/384)		1.6 (6/377)	
	≥ 40 Years		N=435		N=424	
		Positive	3.4 (5/147) (1.1, 7.8)	Not Calculable	2.5 (5/201) (0.8, 5.7)	Not Calculable
		Negative	0.0 (0/288) (0.0, 1.3)		0.0 (0/223) (0.0, 1.6)	
		Prevalence (%)	1.1 (5/435)		1.2 (5/424)	

*18 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

Adjusted clinical performance estimates of the Aptima HPV assay including sensitivity, specificity, PPV, and NPV for the detection of ≥CIN2 and ≥CIN3 are shown in Table 13, as are the estimates for the FDA-approved HPV DNA test. Unadjusted clinical performance estimates are shown in Table 14. The Aptima HPV assay and the FDA-approved HPV DNA test had similar sensitivity, whereas specificity was significantly higher for the Aptima HPV assay (non-overlapping 95% CIs). Predictive value estimates of the Aptima HPV assay were clinically relevant and similar to the estimates for the FDA-approved HPV DNA test. NPVs were similar but for the detection of ≥CIN2, the PPV for the Aptima HPV assay was slightly higher than PPV for the FDA-approved HPV DNA test (4.7% vs 3.7%).

Table 13: NILM ≥ 30 Years Population: Performance of the Aptima HPV Assay and an FDA-approved HPV DNA Test for Detection of ≥CIN2 and ≥CIN3 (Verification-Bias Adjusted Estimates) at Baseline

		Aptima HPV Assay		HPV DNA Test	
	Performance	**Estimate**	**(95% CI)**	**Estimate**	**(95% CI)**
≥CIN2	**Sensitivity (%)**	31.0	(5.9, 56.1)	35.4	(3.8, 66.9)
	Specificity (%)	95.2	(94.8, 95.6)	93.7	(93.2, 94.2)
	PPV (%)	4.7	(2.9, 7.6)	3.7	(2.3, 6.0)
	NPV (%)	99.4	(98.1, 99.8)	99.5	(97.9, 99.9)
	Prevalence (%)	0.9		0.9	
≥CIN3	**Sensitivity (%)**	61.5	(14.0, 100)	56.4	(0.4, 100)
	Specificity (%)	95.2	(94.8, 95.6)	93.6	(93.1, 94.1)
	PPV (%)	3.3	(1.4, 7.6)	2.3	(1.3, 4.1)
	NPV (%)	99.9	(98.4, 100)	99.9	(97.6, 100)
	Prevalence (%)	0.4		0.4	

Table 14: NILM ≥ 30 Years Population: Performance of the Aptima HPV Assay and an FDA-approved HPV DNA Test for Detection of ≥CIN2 and ≥CIN3 (Unadjusted Estimates) at Baseline

		Aptima HPV Assay N=819		HPV DNA Test N=801*	
	Performance	**Estimate**	**(95% CI)**	**Estimate**	**(95% CI)**
≥CIN2	**Sensitivity (%)**	75.0 (15/20)	(53.1, 88.8)	84.2 (16/19)	(62.4, 94.5)
	Specificity (%)	62.6 (500/799)	(59.2, 65.9)	48.7 (381/782)	(45.2, 52.2)
	PPV (%)	4.8 (15/314)	(3.4, 5.8)	3.8 (16/417)	(2.9, 4.4)
	NPV (%)	99.0 (500/505)	(98.1, 99.6)	99.2 (381/384)	(98.1, 99.8)
	Prevalence (%)	2.4 (20/819)		2.4 (19/801)	
≥CIN3	**Sensitivity (%)**	90.9 (10/11)	(62.3, 98.4)	90.9 (10/11)	(62.3, 98.4)
	Specificity (%)	62.4 (504/808)	(59.0, 65.7)	48.5 (383/790)	(45.0, 52.0)
	PPV (%)	3.2 (10/314)	(2.2, 3.7)	2.4 (10/417)	(1.6, 2.7)
	NPV (%)	99.8 (504/505)	(99.1, 100)	99.7 (383/384)	(98.9, 100)
	Prevalence (%)	1.3 (11/819)		1.4 (11/801)	

*18 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

Direct comparison of the Aptima HPV assay and the FDA-approved HPV DNA test demonstrates similar sensitivity and statistically significant improved specificity of the Aptima HPV assay over the FDA-approved HPV DNA test for detection of ≥CIN2 as shown by the ratios of true positive and false positive rates (Table 15 and Table 16, respectively).

Table 15: NILM ≥ 30 Years Population: Ratio of True Positive Rates (Aptima HPV Assay/ FDA-approved HPV DNA Test) for Women with ≥CIN2 (Unadjusted Estimates) at Baseline

		HPV DNA Test		
		Positive	**Negative**	**Total**
Aptima HPV Assay	**Positive**	13	2	15 (78.9%)
	Negative	3	1	4
	Total	16 (84.2%)	3	19
Ratio of True Positive Rates = 0.94 (15/16) (95% CI: 0.67, 1.20)				

Table 16: NILM ≥ 30 Years Population: Ratio of False Positive Rates (Aptima HPV Assay/ FDA-approved HPV DNA Test) for Women with <CIN2 (Unadjusted Estimates) at Baseline

		HPV DNA Test		
		Positive	**Negative**	**Total**
Aptima HPV Assay	**Positive**	223	59	282 (36.1%)
	Negative	178	322	500
	Total	401 (51.3%)	381	782
Ratio of False Positive Rates = 0.70 (282/401) (95% CI: 0.64, 0.77)				

NILM ≥ 30 Years Population: Aptima HPV Assay Clinical Performance After 3 Years of Follow-up

There were 10,854 evaluable women 30 years of age and older with NILM cytology results and valid Aptima HPV assay results at baseline who were eligible for the Follow-up Phase. Of the women without ≥CIN2, 66.9% (7,251/10,834) of women completed a year 1 follow-up Pap visit, 60.2% (6,522/10,825) the year 2, and 58.6% (6,344/10,818) the year 3. Overall, 58.8% (6,380/10,854) of the women completed the study (had ≥CIN2 at baseline or during follow-up, and/or completed required visits).

Of the 10,854 women, 540 (5.0%) had positive Aptima HPV assay results at baseline. Of these 540 women, 263 (48.7%) had either positive or negative 3-year disease status based on cytology or colposcopy/biopsy results. The remaining 10,314 women had negative Aptima HPV assay results at baseline. Of these 10,314 women, 5,943 (57.6%) had either positive or negative 3-year disease status. Of the 6,206 women with 3-year disease status, 47 women had ≥CIN2 including 23 with ≥CIN3; 6,159 women had normal/CIN1 by Consensus Histology Review Panel. The baseline results of the Aptima HPV assay and FDA-approved HPV DNA assay, and the 3-year disease status (includes baseline and follow-up evaluation) by Consensus Histology Review Panel are presented in Table 17.

Table 17: NILM ≥ 30 Years Population: Classification of Women Eligible for the Follow-up Phase by Baseline Aptima HPV Assay, Baseline FDA-approved HPV DNA Test Results, and Disease Status (≥CIN2, ≥CIN3, Unverified) Determined in the Baseline and Follow-up Phases

Aptima HPV 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)	Lost to Follow-up	Indeterminate*
Positive	Positive	360	22	154	15	161	165	19
Positive	Negative	150	2	72	1	73	68	8
Positive	No Result**	30	2	11	1	12	14	3
Negative	Positive	304	6	146	3	149	133	19
Negative	Negative	9,405	14	5,455	3	5,466	3,735	201
Negative	No Result**	605	1	321	0	322	269	14
	Total	10,854	47	6,159	23	6,183	4,384	264

*Women who had abnormal cytology test results during follow-up and who did not have a subsequent Consensus Histology Review Panel result, and women with inadequate cytology at their last visit. 174 women with indeterminate disease status completed follow-up per protocol.
**635 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of cytology specimen.

The 3-year cumulative risk 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 and relative risk estimates for detection of ≥CIN2 and ≥CIN3 are shown in Table 18.

Table 18: NILM ≥ 30 Years Population: 3-Year Cumulative Absolute and Relative Risks* of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an FDA-approved HPV DNA Test at Baseline

		Aptima HPV Assay		HPV DNA Test	
	Assay Result	Absolute Risk (95% CI)	Relative Risk (95% CI)	Absolute Risk (95% CI)	Relative Risk (95% CI)
≥CIN2	Positive	7.39 (5.12, 10.59)	22.55 (12.68, 40.10)	6.42 (4.50, 9.13)	22.71 (12.19, 42.29)
	Negative	0.33 (0.21, 0.51)		0.28 (0.17, 0.47)	
	Prevalence (%)	0.68		0.68	
≥CIN3	Positive	4.66 (2.94, 7.36)	44.12 (16.91, 115.10)	4.14 (2.62, 6.52)	51.33 (17.74, 148.55)
	Negative	0.11 (0.04, 0.25)		0.08 (0.03, 0.22)	
	Prevalence (%)	0.34		0.35	

*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.

The 3-year cumulated prevalence of ≥CIN2 and ≥CIN3 in women with NILM cytology results at baseline were 0.68% and 0.34%, respectively. The relative risk of ≥CIN2 was 22.55 (95% CI: 12.68, 40.10), indicating that a woman who was Aptima HPV assay positive is 22.55 times more likely to have ≥CIN2 than a woman who is Aptima HPV assay negative. The relative risk of ≥CIN3 was 44.12 (95% CI: 16.91, 115.10).

Aptima HPV Assay Agreement with a Composite Comparator

The analytical performance of the Aptima HPV assay was assessed against a composite comparator consisting of an FDA-approved HPV DNA test and a 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 were tested by the comparator assays and compared to the Aptima HPV assay results.[17] In total, 434 samples were tested, with 217 from each of the ASC-US and NILM populations. Women selected included approximately 150 randomly selected women with positive Aptima HPV assay results and <CIN2 disease, approximately 200 randomly selected women with negative Aptima HPV assay results and <CIN2 disease and all women identified with ≥CIN2 disease by consensus histology review at the time of sample selection (when about 80% of the trial enrollment was completed).

For the composite comparator analysis, samples were classified positive if the HPV DNA test and the E6/E7 RT-PCR sequencing test were both positive; negative if the HPV DNA test and the E6/E7 RT-PCR sequencing test were both negative; and indeterminate if the tests were discordant, or if one or both tests returned an invalid or indeterminate result.

Positive and negative percent agreements and associated 95% confidence intervals were calculated. Indeterminate results were not included in the agreement calculations. Results are presented for the ASC-US population (≥ 21 years) and the NILM population (≥ 30 years) in Table 19a and Table 19b, respectively.

Table 19a: ASC-US ≥ 21 Years Population: Aptima HPV Assay Agreement Results with a Composite Comparator (n=217)

		Composite Comparator			Total
		Positive	Negative	Indeterminate	
Aptima HPV Assay	Positive	89	0	27	116
	Negative	2	86	13	101
	Total	91	86	40	217
Positive Percent Agreement: 97.8% (89/91) (95% CI: 92.3, 99.4) **Negative Percent Agreement:** 100.0% (86/86) (95% CI: 95.7, 100)					

Table 19b: NILM ≥ 30 Years Population: Aptima HPV Assay Agreement Results with a Composite Comparator (n=217)

		Composite Comparator			Total
		Positive	Negative	Indeterminate	
Aptima HPV Assay	Positive	55	15	46	116
	Negative	4	63	34	101
	Total	59	78	80	217
Positive Percent Agreement: 93.2% (55/59) (95% CI: 83.8, 97.3) **Negative Percent Agreement:** 80.8% (63/78) (95% CI: 70.7, 88.0)					

Clinical Cutoff Determination for the Aptima HPV Assay

The clinical cutoff for detecting high-grade cervical disease (≥CIN2) for the Aptima HPV assay was established based on the evaluation of approximately 1000 women with ASC-US cytology results enrolled into the ASC-US Study. The method[20] used for selection of the cutoff was chosen to achieve the maximum sensitivity for detecting ≥CIN2 while maintaining a clinically acceptable level of specificity in the ASC-US population. Based on the method described above, the cutoff for the Aptima HPV assay was set at 0.50 S/CO.

Limit of Detection at the Clinical Cutoff

The Limit of Detection (LOD) at the clinical cutoff is the concentration of HPV RNA that gives a positive result (above the clinical cutoff) 95% of the time. The LOD of the Aptima HPV assay was determined by testing dilution panels of in vitro transcripts (IVT) for all 14 high-risk genotypes and 4 HPV-infected cell lines: SiHa, HeLa, MS751 and ME180 (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 two reagent lots for a total of 60 replicates. Testing was performed over 14 days, with 1 to 12 runs performed per day and 5 replicates of a given genotype and concentration tested in each run. The 95% detection limit was calculated from Probit regression analysis of the positivity results for each dilution panel.

The Probit analysis results, Table 20, show that HPV 16, 18, 31, 33, 35, 39, 45, 58, 59, and 68 had 95% detection limits less than 100 copies/reaction; and types 51, 52, 56, and 66 had 95% detection limits between 100 and 300 copies/reaction. The four cell lines tested had 95% detection limits less than 1 cell/reaction.

Table 20: Limit of Detection at Clinical Cutoff of the Aptima HPV Assay

Target	Limit of Detection* (95% CI)
HPV 16	48.7 (36.6 - 72.2)
HPV 18	80.9 (60.4 - 118.4)
HPV 31	18.6 (14.2 - 27.3)
HPV 33	49.1 (37.0 - 71.3)
HPV 35	19.1 (14.2 - 29.1)
HPV 39	24.6 (19.1 - 34.4)
HPV 45	33.8 (25.7 - 49.4)
HPV 51	206.6 (157.5 - 297.7)
HPV 52	266.2 (205.5 - 373.8)
HPV 56	100.1 (81.9 - 129.9)
HPV 58	48.0 (37.3 - 68.7)
HPV 59	49.0 (36.4 - 75.9)
HPV 66	168.7 (129.6 - 241.1)
HPV 68	27.0 (20.3 - 40.1)
SiHa	0.30 (0.24 -0.43)
HeLa	0.18 (0.14 - 0.29)
ME180	0.11 (0.09 - 0.16)
MS751	0.19 (0.14 - 0.33)

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

Assay Precision

Aptima HPV assay precision was evaluated in two studies using the same 20-member panel. Study 1 was conducted at 3 external testing sites and Study 2 was conducted in-house. The panel included 10 HPV-positive members with concentrations at or above the limit of detection of the assay (expected positivity: ≥ 95%), 4 HPV-positive members with concentrations below the limit of detection of the assay (expected positivity: >0% to <25%), and 6 HPV-negative members. HPV-positive panel members were prepared by spiking in vitro RNA transcripts (IVT) into specimen transport medium (STM) or HPV-infected cultured cells (SiHa, HeLa, ME180 and MS751; ATCC, Manassas, Virginia) into PreservCyt Solution.

HPV-negative panel members were prepared with STM or pooled residual ThinPrep liquid cytology specimens.

In Study 1, 2 operators at each of the 3 testing sites (1 instrument per site) performed 1 Aptima HPV assay worklist per day over 3 days for each of 3 reagent lots. Each worklist contained 3 replicates of each of the reproducibility panel members. One hundred sixty-two (162) individual sample tubes were tested for each panel member (3 sites x 1 instrument x 2 operators x 3 lots x 3 worklists 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 21a (panel members with expected positive results) and Table 21b (panel members with expected negative results), along with a summary of the agreement with expected results and analyte S/CO values at the 2.5th, 50th and 97.5th percentiles of the S/CO distribution. The analyte S/CO variability for the panel members with expected positive results is shown in Table 22 for Study 1 and Table 23 for Study 2.

Positive agreement for the HPV-positive panel members with concentrations at or above the limit of detection of the assay ranged from 95.1% to 100% in Study 1 and from 93.2% to 100% in Study 2 for 9 of the 10 panel members. The remaining HPV-positive panel member yielded 77.2% agreement in Study 1 and 79.0% agreement in Study 2, which was lower than expected, but was consistent between the 2 studies. Negative agreement for the HPV-high negative panel members with concentrations below the limit of detection of the assay ranged from 78.8% to 93.8% in Study 1 and from 82.1% to 95.7% in Study 2. Agreement with expected results for the HPV-negative panel members ranged from 96.9% to 100% in Study 1 and from 96.3% to 100% in Study 2.

Table 21a: Aptima HPV Assay Precision Study 1 and 2: Panel Description, Positive Agreement, and Percentile Distribution of Analyte S/CO Values for Panel Members with Expected Positive Results

Panel Description (copies or cells/reaction)	Study 1 (3 testing sites)				Study 2 (1 testing site)			
	% positive agreement (95% CI)	Analyte S/CO Percentile			% positive agreement (95% CI)	Analyte S/CO Percentile		
		2.5th	50th	97.5th		2.5th	50th	97.5th
HPV 16 & HPV 18 IVT (100 copies)	100 (161/161) (97.7, 100)	20.73	23.45	26.31	100 (162/162) (97.7, 100)	20.10	23.21	26.33
SiHa cells (3 cells) & HeLa cells (7.5 cells)	100 (162/162) (97.7, 100)	11.03	15.34	27.97	100 (162/162) (97.7, 100)	12.49	16.49	27.96
HPV 18 IVT (100 copies)	100 (162/162) (97.7, 100)	8.53	11.80	13.87	100 (160/160) (97.7, 100)	9.02	11.89	14.77
HPV 16 IVT (100 copies)	100 (162/162) (97.7, 100)	9.85	10.76	11.64	100 (162/162) (97.7, 100)	9.32	10.91	11.72
MS751 cells (1 cell)	99.4 (161/162) (96.6, 99.9)	6.12	13.89	16.02	96.9 (157/162) (93.0, 98.7)	0.00	14.51	16.61
ME180 cells (0.3 cells)	95.1 (154/162) (90.6, 97.5)	0.00	7.28	9.31	93.2 (151/162) (88.3, 96.2)	0.00	6.50	9.39
HPV 18 IVT (30 copies)	99.4 (161/162) (96.6, 99.9)	3.02	9.31	13.25	100 (162/162) (97.7, 100)	4.29	8.73	13.62
HPV 16 IVT (30 copies)	100 (162/162) (97.7, 100)	8.23	10.94	11.83	97.5 (158/162) (93.8, 99.0)	4.34	10.98	11.88
HeLa cells (2.5 cells)	100 (162/162) (97.7, 100)	6.64	12.88	15.84	95.6 (152/159) (91.2, 97.9)	0.00	12.85	16.83
SiHa cells (1 cell)*	77.2 (125/162) (70.1, 83.0)	0.00	10.50	12.13	79.0 (128/162) (72.1, 84.6)	0.00	10.54	11.77

IVT = in vitro transcript. IVT was spiked into STM and cells were spiked into PreservCyt Solution.
*Expected % positive agreement ~95%; observed lower possibly due to manufacturing variability of the panel member.

Table 21b: Aptima HPV Assay Precision Study 1 and 2: Panel Description, Negative Agreement, and Percentile Distribution of Analyte S/CO Values for Panel Members with Expected Negative Results

Panel Description (copies or cells/reaction)	Study 1 (3 testing sites)				Study 2 (1 testing site)			
	% negative agreement (95% CI)	Analyte S/CO Percentile			% negative agreement (95% CI)	Analyte S/CO Percentile		
		2.5th	50th	97.5th		2.5th	50th	97.5th
HPV 18 IVT (1 copy)*	78.8 (126/160) (71.8, 84.4)	0.00	0.00	4.55	83.3 (135/162) (76.8, 88.3)	0.00	0.00	5.58
HPV 16 IVT (1 copy)*	80.9 (131/162) (74.1, 86.2)	0.00	0.00	10.70	88.3 (143/162) (82.4, 92.4)	0.00	0.00	11.33
HeLa cells (0.05 cells)*	79.0 (128/162) (72.1, 84.6)	0.00	0.00	10.44	82.1 (133/162) (75.5, 87.2)	0.00	0.00	11.63
SiHa cells (0.03 cells)*	93.8 (152/162) (89.0, 96.6)	0.00	0.00	10.71	95.7 (155/162) (91.4, 97.9)	0.00	0.00	6.87
STM Lot 1	100 (162/162) (97.7, 100)	0.00	0.00	0.05	100 (162/162) (97.7, 100)	0.00	0.00	0.00
STM Lot 2	99.4 (160/161) (96.6, 99.9)	0.00	0.00	0.19	100 (162/162) (97.7, 100)	0.00	0.00	0.00
STM Lot 3	99.4 (161/162) (96.6, 99.9)	0.00	0.00	0.11	99.4 (161/162) (96.6, 99.9)	0.00	0.00	0.00
ThinPrep Pool 1	97.5 (158/162) (93.8, 99.0)	0.00	0.00	0.31	97.5 (158/162) (93.8, 99.0)	0.00	0.00	0.33
ThinPrep Pool 2	96.9 (157/162) (93.0, 98.7)	0.00	0.00	0.68	96.3 (156/162) (92.2, 98.3)	0.00	0.00	1.55
ThinPrep Pool 3	100 (162/162) (97.7, 100)	0.00	0.00	0.16	99.4 (161/162) (96.6, 99.9)	0.00	0.00	0.00

IVT = in vitro transcript; STM = specimen transport medium. IVT was spiked into STM and cells were spiked into PreservCyt Solution.
*Expected % negative agreement > 75% and < 100%.

Table 22: Aptima HPV Assay Precision Study 1: Signal Variability for Panel Members With Expected Positive Results

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 & HPV 18 IVT (100 copies)	161^	23.43	0.10	0.4	0.10	0.4	0.94	4.0	0.00	0.0	1.64	7.0	1.89	8.1
SiHa cells (3 cells) & HeLa cells (7.5 cells)	162	17.85	0.00	0.0	1.44	8.1	0.00	0.0	0.55	3.1	5.10	28.6	5.33	29.9
HPV 18 IVT (100 copies)	162	11.82	0.00	0.0	0.00	0.0	0.76	6.4	0.10	0.9	1.19	10.1	1.42	12.0
HPV 16 IVT (100 copies)	162	10.76	0.17	1.5	0.00	0.0	0.12	1.1	0.28	2.6	0.33	3.1	0.48	4.5
MS751 cells (1 cell)	162	13.30	0.28	2.1	0.00	0.0	1.03	7.8	0.94	7.1	2.15	16.2	2.58	19.4
ME180 cells (0.3 cells)	162	6.51	0.21	3.2	0.00	0.0	0.56	8.6	0.36	5.5	2.36	36.2	2.46	37.7
HPV 18 IVT (30 copies)	162	9.00	0.66	7.3	0.00	0.0	0.65	7.2	0.75	8.3	2.28	25.3	2.57	28.5
HPV 16 IVT (30 copies)	162	10.76	0.09	0.8	0.00	0.0	0.14	1.3	0.41	3.8	0.90	8.4	1.01	9.3
HeLa cells (2.5 cells)	162	12.36	0.00	0.0	0.41	3.3	0.39	3.1	0.00	0.0	2.28	18.4	2.35	19.0
SiHa cells (1 cell)	162	7.47	0.27	3.7	0.97	13.0	0.00	0.0	0.00	0.0	4.75	63.6	4.85	65.0

CV = coefficient of variation; IVT = in vitro transcript; SD = standard deviation
^One sample had an invalid Aptima HPV assay result and was not included in the analyses.
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 Assay Precision Study 2: Signal Variability for Panel Members with Expected Positive Results

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 & HPV 18 IVT (100 copies)	162	23.21	0.35	1.5	0.55	2.3	0.79	3.4	0.80	3.4	1.47	6.3	1.96	8.4
SiHa cells (3 cells) & HeLa cells (7.5 cells)	162	18.62	0.00	0.0	1.74	9.3	0.00	0.0	3.46	18.6	3.72	20.0	5.37	28.9
HPV 18 IVT (100 copies)	160^	11.92	0.07	0.6	0.20	1.6	0.83	7.0	0.43	3.6	1.34	11.3	1.65	13.8
HPV 16 IVT (100 copies)	162	10.83	0.00	0.0	0.14	1.3	0.00	0.0	0.24	2.2	0.66	6.1	0.72	6.6
MS751 cells (1 cell)	162	13.63	0.00	0.0	0.58	4.3	0.00	0.0	2.50	18.4	2.07	15.2	3.30	24.2
ME180 cells (0.3 cells)	162	5.81	0.00	0.0	0.63	10.8	0.54	9.4	2.15	36.9	1.73	29.7	2.88	49.5
HPV 18 IVT (30 copies)	162	8.84	0.39	4.4	0.53	6.0	0.70	7.9	1.02	11.5	1.89	21.4	2.35	26.6
HPV 16 IVT (30 copies)	162	10.50	0.00	0.0	0.13	1.3	0.21	2.0	1.57	14.9	1.18	11.2	1.98	18.8
HeLa cells (2.5 cells)	159^	11.96	0.61	5.1	1.02	8.5	0.00	0.0	2.84	23.8	1.98	16.6	3.66	30.6
SiHa cells (1 cell)	162	7.43	0.93	12.5	0.00	0.0	0.69	9.3	1.82	24	4.22	56.8	4.74	63.8

CV = coefficient of variation; IVT = in vitro transcript; SD = standard deviation
^Five samples had invalid Aptima HPV assay results (2 for HPV 18 IVT (100 copies), 3 for HeLa cells (2.5 cells)) 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 6-member panel of pooled clinical ThinPrep liquid cytology specimens. Six unique pools of residual HPV-negative ThinPrep liquid cytology specimens were prepared as the matrix, two of which were tested as HPV-negative panel members. Four unique pools of HPV-positive ThinPrep liquid cytology specimens were used to prepare the low (n=2) and high (n=2) HPV-positive panel members. The low positive panel members had concentrations at the limit of detection of the assay (expected positivity: ≥ 95% determined for each individual HPV-positive pool from testing serial dilutions of the pools). The high positive panel members had concentrations at 1-2 logs above the estimated limit of detection for each individual HPV positive pool (expected positivity: 100% positivity). Each panel member was transferred (1 mL) into an Aptima Specimen Transfer tube containing STM on the day of testing. Testing was conducted in-house by 2 operators using 1 reagent lot, 3 instruments, over 6 days (3 days for each operator), testing 2 runs per day in which the panel was tested in duplicate.

The panel members are described in Table 24, along with a summary of the agreement with expected results and analyte S/CO values at the 2.5th, 50th, and 97.5th percentiles of the signal distribution. The analyte S/CO variability for the panel members with expected positive results is shown in Table 25.

Table 24: Aptima HPV Assay Precision Study 3: Panel Description, Percent Agreement, and Percentile Distribution of Analyte S/CO Values

Panel Description	% agreement (95% CI)	Analyte S/CO Percentile		
		2.5th	50th	97.5th
Low positive 1	98.6 (71/72) (92.5, 99.8)	1.47	10.08	19.32
Low positive 2	100 (72/72) (94.9, 100)	1.52	10.28	19.11
High positive 1	100 (72/72) (94.9, 100)	12.64	23.13	32.35
High positive 2	100 (72/72) (94.9, 100)	13.34	24.73	31.23
Negative 1	98.6 (71/72) (92.5, 99.8)	0.00	0.00	0.27
Negative 2	94.4 (68/72) (86.6, 97.8)	0.00	0.00	0.65

Table 25: Aptima HPV Assay Precision Study 3: Signal Analysis for Panel Members with Expected Positive Results

Panel Description	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 (%)
Low positive 1	72	9.79	0.00	0.0	0.00	0.0	0.00	0.0	2.23	22.8	2.98	30.4	3.72	38.0
Low positive 2	72	10.49	0.00	0.0	2.21	21.0	0.94	9.0	3.70	35.3	2.74	26.1	5.19	49.5
High positive 1	72	22.70	1.28	5.6	0.00	0.0	0.10	0.5	3.03	13.3	3.71	16.4	4.96	21.9
High positive 2	72	23.90	0.00	0.0	0.00	0.0	0.00	0.0	2.93	12.3	2.96	12.4	4.17	17.4

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 assay was evaluated with PreservCyt Solution media diluted 1:2.9 into STM and spiked with cultured bacteria, yeast, or fungi; cultured virus; or low-risk HPV *in vitro* transcripts. The organisms and test concentrations are identified in Table 26. The study criteria for assessing the effect of the presence of microorganism on the specificity of the assay were based on positivity. Cross-reactivity was observed with low-risk HPV genotypes 26, 67, 70, and 82, but not with any of the other organisms tested.

Table 26: 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	$1x10^8$ CFU/mL	*Listeria monocytogenes*	$1x10^8$ CFU/mL
Actinomyces israelii	$1x10^8$ CFU/mL	*Micrococcus luteus*	$1x10^8$ CFU/mL
Alcaligenes faecalis	$1x10^8$ CFU/mL	*Mobiluncus curtisii*	$2x10^7$ CFU/mL
Atopobium vaginae	$5x10^7$ CFU/mL	*Mycobacterium smegmatis*	$1x10^8$ CFU/mL
Bacillus cereus	$1x10^8$ CFU/mL	*Mycoplasma fermentans*	$5x10^7$ CFU/mL
Bacteroides fragilis	$1x10^8$ CFU/mL	*Mycoplasma genitalium*	$1x10^8$ CFU/mL
Bacteroides ureolyticus	$1x10^8$ CFU/mL	*Mycoplasma hominis*	$5x10^7$ CFU/mL
Bifidobacterium adolescentis	$1x10^8$ CFU/mL	*Neisseria gonorrhoeae*	$1x10^8$ CFU/mL
Bifidobacterium breve	$1x10^8$ CFU/mL	*Neisseria gonorrhoeae* and *Chlamydia trachomatis*	$2.5x10^7$ CFU/mL $2.3x10^5$ $TCID_{50}$/mL
Campylobacter fetus-fetus	$1x10^8$ CFU/mL	*Neisseria meningitidis*	$1x10^8$ CFU/mL
Chlamydia trachomatis	$3.2x10^5$ $TCID_{50}$/mL	*Peptoniphilus lacrimalis*	$1x10^8$ CFU/mL
Clostridium difficile	$6x10^7$ CFU/mL	*Peptostreptococcus anaerobius*	$1x10^8$ CFU/mL
Clostridium perfringens	$1x10^8$ CFU/mL	*Propionibacterium acnes*	$1x10^8$ CFU/mL
Corynebacterium genitalium	$1x10^8$ CFU/mL	*Proteus mirabilis*	$1x10^8$ CFU/mL
Corynebacterium xerosis	$1x10^8$ CFU/mL	*Proteus vulgaris*	$1x10^8$ CFU/mL
Enterobacter cloacae	$1x10^8$ CFU/mL	*Providencia stuartii*	$1x10^8$ CFU/mL
Enterococcus faecalis	$1x10^8$ CFU/mL	*Pseudomonas aeruginosa*	$1x10^8$ CFU/mL
Escherichia coli	$1x10^8$ CFU/mL	*Ruminococcus productus*	$1x10^8$ CFU/mL
Finegoldia magna	$1x10^8$ CFU/mL	*Serratia marcescens*	$1x10^8$ CFU/mL
Fusobacterium nucleatum	$1x10^8$ CFU/mL	*Staphylococcus aureus*	$1x10^8$ CFU/mL
Gardnerella vaginalis	$1x10^8$ CFU/mL	*Staphylococcus epidermidis*	$1x10^8$ CFU/mL
Haemophilus ducreyi	$1x10^8$ CFU/mL	*Staphylococcus saprophyticus*	$1x10^8$ CFU/mL
Klebsiella pneumoniae	$1x10^8$ CFU/mL	*Streptococcus agalactiae*	$1x10^8$ CFU/mL
Lactobacillus acidophilus	$1x10^8$ CFU/mL	*Streptococcus pyogenes*	$1x10^8$ CFU/mL
Lactobacillus crispatus	$1x10^8$ CFU/mL	*Streptococcus sanguinis*	$1x10^8$ CFU/mL
Lactobacillus delbrueckii ssp. bulgaricus	$1x10^8$ CFU/mL	*Ureaplasma urealyticum*	$1x10^8$ CFU/mL

Table 26: Analytical Specificity Panel: Organisms and Concentration with No Cross-Reactivity

Organism	Test Concentration with No Cross-Reactivity	Organism	Test Concentration with No Cross-Reactivity
Lactobacillus jensenii	$1x10^8$ CFU/mL		
Yeast/protozoa			
Candida albicans	$1x10^8$ CFU/mL	*Trichomonas vaginalis*	$1x10^7$ cells/mL
Viruses			
Adenovirus 2	$1x10^7$ vp/mL	Herpes simplex virus 1	$2.5x10^5$ $TCID_{50}$/mL
Cytomegalovirus	$5.6x10^2$ $TCID_{50}$/mL	Herpes simplex virus 2	$5x10^4$ $TCID_{50}$/mL
Epstein-Barr virus	$4.3x10^6$ vp/mL	SV40	$1.2 x10^4$ $TCID_{50}$/mL
HIV-1	$1.0x10^6$ copies/mL		
Non-targeted HPV genotypes			
HPV 6	$2.5x10^6$ copies/mL	HPV 61	$2.5x10^6$ copies/mL
HPV 11	$2.5x10^6$ copies/mL	**HPV 67**	1 copy/mL
HPV 26	2.5 copies/mL	HPV 69	$2.5x10^6$ copies/mL
HPV 30	$2.5x10^6$ copies/mL	**HPV 70**	1 copy/mL
HPV 34	$2.5x10^6$ copies/mL	HPV 71	$2.5x10^6$ copies/mL
HPV 42	$2.5x10^6$ copies/mL	HPV 73	$2.5x10^6$ copies/mL
HPV 43	$2.5x10^6$ copies/mL	HPV 81	$2.5x10^6$ copies/mL
HPV 44	$2.5x10^6$ copies/mL	**HPV 82**	1 copy/mL
HPV 53	$2.5x10^6$ copies/mL	HPV 85	$2.5x10^6$ copies/mL
HPV 54	$2.5x10^6$ copies/mL		

vp = viral particles CFU = colony forming units $TCID_{50}$ = tissue culture infective dose 50

Note: *Bold indicates types where cross-reactivity (> 5% positivity) was observed when tested at concentrations greater than that noted in the table.*

The analytical sensitivity of the Aptima HPV assay in the presence of microorganisms was evaluated with the same panel described in Table 26, which was also spiked with a low concentration of HPV infected SiHa cells (1 cell per reaction). The study criteria for assessing the effect of the presence of microorganism on the sensitivity of the assay were based on positivity. The sensitivity of the Aptima HPV assay was not affected by any of the organisms tested.

Interference

The substances described in Table 27 were individually spiked into PreservCyt Solution at 1% and 10% v/v or w/v, diluted with STM and then tested in the Aptima HPV assay. All substances were tested in the presence and absence of HPV infected cultured cells (SiHa, 3 cells/reaction). Interference was observed with two of the seven lubricants that contained Polyquaternium 15, and one of the five anti-fungal medications that contained tioconazole. Interference was not observed with any of other substances tested.

Table 27: Substances Tested for Possible Interference with the Aptima HPV Assay

Product Category	Product Brand or Type	Highest Concentration* Tested that Did Not Interfere with Assay Performance
Lubricant	KY Sensual Mist	10% v/v
	KY Warming Jelly	10% w/v
	KY Warming Liquid	10% v/v
	CVS Brand Personal Lubricant	10% w/v
	Target Brand Warming Massage Lotion and Personal Lubricant	10% v/v
	Astroglide Personal Lubricant	0.3% w/v (0.075% w/v test sample)
	Target Brand Lubricating Liquid	0.1% v/v (0.025% v/v test sample)
Spermicide	Gynol II Vaginal Contraceptive Original Formula	10% w/v
	Gynol II Vaginal Contraceptive Extra Strength	10% w/v
	Delfen Vaginal Contraceptive Foam	10% w/v
	Encare Vaginal Contraceptive	10% w/v
	Conceptrol Vaginal Contraceptive	10% w/v
Anti-fungal/ Anti-Itch Medication	Vagisil Maximum Strength	10% w/v
	Monistat Soothing Care	10% w/v
	Monistat 3 Combination Pack	10% w/v
	Target Brand Tioconazole 1	0.3% w/v (0.075% w/v test sample)
	Target Brand Miconazole 3	10% w/v
Glacial Acetic Acid	EMD M/N AX0073-11	10% v/v
Whole Blood	whole blood	10% v/v

*Concentration in the PreservCyt Solution specimen; test sample concentration is 1/4 the concentration due to transfer of PreservCyt Solution specimen into transfer tube containing STM.

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 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 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 28).

Table 28: 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	98.7	0.0	N/A
		(83.9, 100.0)	(93.2, 99.8)	(0.0, 79.3)	
		20/20	78/79	0/1	
	Negative Percent Agreement	N/A	0.0	97.4	100.0
			(0.0, 79.3)	(86.8, 99.5)	(94.0, 100.0)
			0/1	38/39	60/60
Total		20	80	40	60

CI = Confidence Interval

Panther System Expected Results: Prevalence of High-Risk HPV mRNA

The prevalence of high-risk HPV infection varies widely and is influenced by several factors, for which age is the greatest contributor.[15,16] Many studies have investigated HPV prevalence as determined by the detection of HPV DNA, however few studies report prevalence based on detection of HPV oncogenic mRNA. Women from a variety of clinical sites (n=18) representing a wide geographic distribution and a diverse population (10 states within the United States) were enrolled in a prospective clinical study known as the CLEAR trial.[17] As determined by the Aptima HPV assay on the Panther System, the prevalence of HPV mRNA-positive samples observed in the clinical trial was categorized overall, by age group, and by testing site. Results are shown in Table 29 for the ASC-US (atypical squamous cells of undetermined significance) and the NILM (negative for intraepithelial lesion or malignancy) populations.

Table 29: High-risk HPV mRNA Prevalence by Age Group, Testing Site, and All Combined

	Positivity Rate % (x/n)	
	ASC-US Population (≥ 21 Years)	**NILM Population (≥ 30 Years)**
All	42.3 (404/956)	4.7 (512/10,860)
Age Group (years)		
21 to 29	60.0 (251/418)	N/A
30 to 39	38.1 (101/265)	6.8 (286/4192)
≥ 40	19.0 (52/273)	3.4 (226/6668)
Testing Site		
1	41.5 (134/323)	3.7 (304/8286)
2	43.1 (137/318)	9.2 (118/1285)
3	42.2 (133/315)	7.0 (90/1289)

N/A = Not Applicable

Aptima HPV Assay on the Panther System Clinical Study Design

The Aptima HPV assay on the Panther system was evaluated using residual referral cytology specimens collected from consenting women during the prospective, multicenter US clinical study known as the CLEAR trial.[17]

CLEAR Trial – Baseline Evaluation

The CLEAR trial was conducted to determine the clinical performance of the Aptima HPV assay on the Tigris 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 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. The NILM Study was designed to support the adjunctive screening claim for women 30 years and older, since women in this age range with cytology results greater than ASC-US should proceed to colposcopy regardless of their HPV status.[14]

Women from 18 clinical sites, primarily obstetrics/gynecology clinics, which covered a wide geographic distribution and a diverse population, were enrolled. Eligible women were assigned to the ASC-US Study or NILM Study based on their referral ThinPrep liquid based cytology specimen. At baseline, residual referral specimens from women in the ASC-US Study and in the NILM Study were initially tested with both the Aptima HPV assay on the Tigris System and an FDA-approved HPV DNA test. The specimens were then archived and stored at -70°C until they were tested with the Aptima HPV assay on the Panther System.

At baseline of the CLEAR trial (Baseline Phase), all women in the ASC-US Study were referred to colposcopy, regardless of their HPV 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. The randomly selected women who were negative for both assays were included to correct for verification bias with adjusted performance estimates generated using a multiple imputation method. An ECC biopsy was obtained from each woman who attended colposcopy. Punch biopsies were obtained from visible lesions only (directed 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 woman's HPV status. They were also masked to cytology status, as well as each other's histology diagnoses. If all 3 pathologists disagreed, all 3 pathologists reviewed the 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 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. Clinical performance

of the FDA-approved HPV DNA test was also determined for direct comparison to the Aptima HPV assay results.

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 NILM study 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 results with the cumulative 3-year risk of cervical disease in women with baseline negative Aptima HPV 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 assay on the Panther system 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 Assay Clinical Performance

In total, there were 1252 women 21 years of age and older with ASC-US cytology results enrolled in the ASC-US Study, of these, 294 women were withdrawn. The remaining 958 women were eligible for testing on the Panther System. Two women had missing samples and 19 had an undetermined disease diagnosis; all were excluded from analysis. The remaining 937 evaluable women were 21 years of age and older with ASC-US cytology results, Aptima HPV assay results on the Panther System, and conclusive disease status. Ninety-one (91) women had ≥CIN2 and forty-one (41) had ≥CIN3. Prevalence of ≥CIN2 and ≥CIN3 in evaluable women with ASC-US cytology results were 9.7% and 4.4%, respectively. The results of the Aptima HPV assay by the Consensus Histology Review Panel diagnoses are presented in Table 30.

Table 30: ASC-US ≥ 21 Years Population: Results of the Aptima HPV Assay by Consensus Histology Review Panel Diagnosis

Aptima HPV Assay Result*	HPV DNA Test	Consensus Histology Review Panel Diagnosis						
		Undetermined**	Normal	CIN1	CIN2	CIN3	Cancer	Total
Positive	Positive	6	178	110	40	32	1	367
Positive	Negative	0	5	2	0	2	0	9
Positive	No Result***	0	15	11	0	2	0	28
Negative	Positive	0	39	15	3	3	0	60
Negative	Negative	10	372	53	7	1	0	443
Negative	No Result***	3	39	7	0	0	0	49
Total		19	648	198	50	40	1****	956

*All samples had final valid results (upon initial testing or after resolution of initial invalids per procedure).
**19 subjects 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).
***77 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.
****One subject had adenocarcinoma in situ (AIS).

Clinical performance estimates of the Aptima HPV assay including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the detection of ≥CIN2 and ≥CIN3 based on evaluating all biopsies and including only directed biopsies are shown in Table 31, as are the estimates for the FDA-approved HPV DNA test.

Table 31: ASC-US ≥ 21 Years Population: Performance of the Aptima HPV Assay and an FDA-approved HPV DNA Test for Detection of ≥CIN2 and ≥CIN3

		Aptima HPV Assay N=937		HPV DNA Test N=863*	
	Performance	**Estimate**	**(95% CI)**	**Estimate**	**(95% CI)**
≥CIN2	**All Biopsies**				
	Sensitivity (%)	84.6 (77/91)	(75.8, 90.6)	88.8 (79/89)	(80.5, 93.8)
	Specificity (%)	62.1 (525/846)	(58.7, 65.3)	55.8 (432/774)	(52.3, 59.3)
	PPV (%)	19.3 (77/398)	(17.3, 21.2)	18.8 (79/421)	(17.0, 20.4)
	NPV (%)	97.4 (525/539)	(96.0, 98.5)	97.7 (432/442)	(96.2, 98.8)
	Prevalence (%)	9.7 (91/937)		10.3 (89/863)	
	Directed Biopsies**				
	Sensitivity (%)	90.0 (54/60)	(79.9, 95.3)	93.2 (55/59)	(83.8, 97.3)
	Specificity (%)	60.8 (531/874)	(57.5, 63.9)	54.5 (437/802)	(51.0, 57.9)
	PPV (%)	13.6 (54/397)	(12.0, 15.0)	13.1 (55/420)	(11.7, 14.2)
	NPV (%)	98.9 (531/537)	(97.8, 99.6)	99.1 (437/441)	(97.9, 99.7)
	Prevalence (%)	6.4 (60/934)		6.9 (59/861)	
≥CIN3	**All Biopsies**				
	Sensitivity (%)	90.2 (37/41)	(77.5, 96.1)	92.3 (36/39)	(79.7, 97.3)
	Specificity (%)	59.7 (535/896)	(56.5, 62.9)	53.3 (439/824)	(49.9, 56.7)
	PPV (%)	9.3 (37/398)	(8.0, 10.3)	8.6 (36/421)	(7.4, 9.4)
	NPV (%)	99.3 (535/539)	(98.3, 99.8)	99.3 (439/442)	(98.3, 99.8)
	Prevalence (%)	4.4 (41/937)		4.5 (39/863)	
	Directed Biopsies**				
	Sensitivity (%)	93.1 (27/29)	(78.0, 98.1)	96.4 (27/28)	(82.3, 99.4)
	Specificity (%)	59.1 (535/906)	(55.8, 62.2)	52.8 (440/834)	(49.4, 56.1)
	PPV (%)	6.8 (27/398)	(5.7, 7.5)	6.4 (27/421)	(5.5, 7.0)
	NPV (%)	99.6 (535/537)	(98.8, 100)	99.8 (440/441)	(98.9, 100)
	Prevalence (%)	3.1 (29/935)		3.2 (28/862)	

*74 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

**Consensus histology result was derived using only results from directed biopsies. Women with no directed biopsies reflect a normal colposcopy and are included in these analyses as non-diseased (<CIN2 or <CIN3, as appropriate). A consensus was not always reached when only directed biopsies were included.

When evaluating all biopsies, clinical sensitivity estimates of the Aptima HPV assay and the FDA-approved HPV DNA test for the detection of ≥CIN2 and ≥CIN3, where both assay results are available, were similar (differences in sensitivity estimates were not statistically significant). For ≥CIN2 the sensitivity difference was -4.5% (95% CI: -12.2%, 2.5%). Clinical specificity estimates of the Aptima HPV assay for the detection of ≥CIN2 and ≥CIN3 were higher than those of the FDA-approved HPV DNA test (differences in specificity estimates were statistically significant). For ≥CIN2, the specificity difference was 6.1% (95% CI: 4.2%, 8.2%). NPVs were similar but for the detection of ≥CIN2, the PPV for the Aptima HPV assay was slightly higher than PPV for the FDA-approved HPV DNA test (19.3% vs 18.8%).

Of the 91 ≥CIN2 cases, 60 (65.9%) were identified in directed biopsies and 31 (34.1%) were identified from random and/or ECC biopsies (i.e., not in directed biopsies). These findings are comparable to results from published studies, in which approximately 25% to 40% of ≥CIN2 cases were identified from random and/or ECC biopsy specimens only.[18,19] Using only directed biopsies to determine disease status (assuming women with no directed biopsies had normal histology results because no visible lesions were present), prevalence of ≥CIN2 and ≥CIN3 in the study were 6.4% and 3.1%, respectively. The clinical sensitivity estimates for the detection of ≥CIN2 and ≥CIN3 were higher for both tests using directed biopsies only than estimates calculated using all biopsies. For both assays, clinical specificity using only directed biopsies was similar to the specificity obtained with all biopsies included. Accordingly, when using only directed biopsies, the Aptima HPV assay specificity was significantly higher than that of the FDA-approved HPV DNA test.

Clinical performance estimates of the Aptima HPV assay and the FDA-approved HPV DNA test are shown by age group in Table 32 and Table 33 (≥CIN2 and ≥CIN3, respectively, based on evaluating all biopsies).

Table 32: ASC-US ≥ 21 Years Population: Performance of the Aptima HPV Assay and an FDA-approved HPV DNA Test for Detection of ≥CIN2 by Age Group

		Aptima HPV Assay N=937		HPV DNA Test N=863*	
	Performance	**Estimate**	**(95% CI)**	**Estimate**	**(95% CI)**
21 to 29 Years		N=415		N=389	
	Sensitivity (%)	88.5 (54/61)	(78.2, 94.3)	94.9 (56/59)	(86.1, 98.3)
	Specificity (%)	44.9 (159/354)	(39.8, 50.1)	35.5 (117/330)	(30.5, 40.8)
	PPV (%)	21.7 (54/249)	(19.3, 23.9)	20.8 (56/269)	(19.0, 22.5)
	NPV (%)	95.8 (159/166)	(92.3, 98.1)	97.5 (117/120)	(93.6, 99.4)
	Prevalence (%)	14.7 (61/415)		15.2 (59/389)	
30 to 39 Years		N=261		N=238	
	Sensitivity (%)	85.0 (17/20)	(64.0, 94.8)	80.0 (16/20)	(58.4, 91.9)
	Specificity (%)	66.4 (160/241)	(60.2, 72.1)	61.9 (135/218)	(55.3, 68.1)
	PPV (%)	17.3 (17/98)	(13.1, 21.1)	16.2 (16/99)	(11.8, 19.8)
	NPV (%)	98.2 (160/163)	(95.7, 99.6)	97.1 (135/139)	(94.1, 99.1)
	Prevalence (%)	7.7 (20/261)		8.4 (20/238)	
≥ 40 Years		N=261		N=236	
	Sensitivity (%)	60.0 (6/10)	(31.3, 83.2)	70.0 (7/10)	(39.7, 89.2)
	Specificity (%)	82.1 (206/251)	(76.9, 86.3)	79.6 (180/226)	(73.9, 84.4)
	PPV (%)	11.8 (6/51)	(5.6, 17.7)	13.2 (7/53)	(6.9, 18.7)
	NPV (%)	98.1 (206/210)	(96.6, 99.4)	98.4 (180/183)	(96.6, 99.6)
	Prevalence (%)	3.8 (10/261)		4.2 (10/236)	

*74 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

Table 33: ASC-US ≥ 21 Years Population: Performance of the Aptima HPV Assay and an FDA-approved HPV DNA Test for Detection of ≥CIN3 by Age Group

		Aptima HPV Assay N=937		HPV DNA Test N=863*	
	Performance	**Estimate**	**(95% CI)**	**Estimate**	**(95% CI)**
21 to 29 Years		N=415		N=389	
	Sensitivity (%)	96.3 (26/27)	(81.7, 99.3)	100 (25/25)	(86.7, 100)
	Specificity (%)	42.5 (165/388)	(37.7, 47.5)	33.0 (120/364)	(28.3, 38.0)
	PPV (%)	10.4 (26/249)	(9.0, 11.5)	9.3 (25/269)	(8.2, 10.0)
	NPV (%)	99.4 (165/166)	(97.2, 100)	100 (120/120)	(97.5, 100)
	Prevalence (%)	6.5 (27/415)		6.4 (25/389)	
30 to 39 Years		N=261		N=238	
	Sensitivity (%)	88.9 (8/9)	(56.5, 98.0)	77.8 (7/9)	(45.3, 93.7)
	Specificity (%)	64.3 (162/252)	(58.2, 69.9)	59.8 (137/229)	(53.4, 66.0)
	PPV (%)	8.2 (8/98)	(5.0, 10.1)	7.1 (7/99)	(4.0, 9.2)
	NPV (%)	99.4 (162/163)	(97.6, 100)	98.6 (137/139)	(96.4, 99.8)
	Prevalence (%)	3.4 (9/261)		3.8 (9/238)	
≥ 40 Years		N=261		N=236	
	Sensitivity (%)	60.0 (3/5)	(23.1, 88.2)	80.0 (4/5)	(37.6, 96.4)
	Specificity (%)	81.3 (208/256)	(76.0, 85.6)	78.8 (182/231)	(73.1, 83.6)
	PPV (%)	5.9 (3/51)	(1.6, 9.7)	7.5 (4/53)	(2.9, 10.7)
	NPV (%)	99.0 (208/210)	(98.0, 99.9)	99.5 (182/183)	(98.2, 100)
	Prevalence (%)	1.9 (5/261)		2.1 (5/236)	

*74 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

The absolute risk of disease (≥CIN2 and ≥CIN3, based on evaluating all biopsies) by Aptima HPV assay result and the relative risk of disease for positive versus negative Aptima HPV assay results are shown in Table 34, as are the estimates for the FDA-approved HPV DNA test. The relative risk of ≥CIN2 was 7.4 (95% CI: 4.3, 13.0), indicating that a woman who was Aptima HPV assay positive was 7.4 times as likely to have ≥CIN2 than a woman who was Aptima HPV assay negative. The relative risk of ≥CIN3 was 12.5 (95% CI: 4.5, 34.9).

Table 34: ASC-US ≥ 21 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an FDA-approved HPV DNA Test

		Aptima HPV Assay N=937		HPV DNA test N=863*	
	Assay Result	Absolute Risk (95% CI)	Relative Risk (95% CI)	Absolute Risk (95% CI)	Relative Risk (95% CI)
≥CIN2	Positive	19.3 (77/398) (17.3, 21.2)	7.4 (4.3, 13.0)	18.8 (79/421) (17.0, 20.4)	8.3 (4.4, 15.8)
	Negative	2.6 (14/539) (1.5, 4.0)		2.3 (10/442) (1.2, 3.8)	
	Prevalence (%)	9.7 (91/937)		10.3 (89/863)	
≥CIN3	Positive	9.3 (37/398) (8.0, 10.3)	12.5 (4.5, 34.9)	8.6 (36/421) (7.4, 9.4)	12.6 (3.9, 40.6)
	Negative	0.7 (4/539) (0.2, 1.7)		0.7 (3/442) (0.2, 1.7)	
	Prevalence (%)	4.4 (41/937)		4.5 (39/863)	

*74 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

Absolute and relative risk estimates of disease (≥CIN2 and ≥CIN3, based on evaluating all biopsies) for the Aptima HPV assay and the FDA-approved HPV DNA test are shown by age group in Table 35.

Table 35: ASC-US ≥ 21 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an FDA-approved HPV DNA Test by Age Group

			Aptima HPV Assay N=937		HPV DNA Test N=863*	
	Age	Assay Result	Absolute Risk (95% CI)	Relative Risk (95% CI)	Absolute Risk (95% CI)	Relative Risk (95% CI)
≥CIN2	21 to 29 Years		N=415		N=389	
		Positive	21.7 (54/249) (19.3, 23.9)	5.1 (2.4, 11.0)	20.8 (56/269) (19.0, 22.5)	8.3 (2.7, 26.1)
		Negative	4.2 (7/166) (1.9, 7.7)		2.5 (3/120) (0.6, 6.4)	
		Prevalence (%)	9.7 (61/415)		15.2 (59/389)	
	30 to 39 Years		N=261		N=238	
		Positive	17.3 (17/98) (13.1, 21.1)	9.4 (2.8, 31.3)	16.2 (16/99) (11.8, 19.8)	5.6 (1.9, 16.3)
		Negative	1.8 (3/163) (0.4, 4.3)		2.9 (4/139) (0.9, 5.9)	
		Prevalence (%)	7.7 (20/261)		8.4 (20/238)	
	≥ 40 Years		N=261		N=236	
		Positive	11.8 (6/51) (5.6, 17.7)	6.2 (1.8, 21.1)	13.2 (7/53) (6.9, 18.7)	8.1 (2.2, 30.1)
		Negative	1.9 (4/210) (0.6, 3.4)		1.6 (3/183) (0.4, 3.4)	
		Prevalence (%)	3.8 (10/261)		4.2 (10/236)	
≥CIN3	21 to 29 Years		N=415		N=389	
		Positive	10.4 (26/249) (9.0, 11.5)	17.3 (2.4, 127)	9.3 (25/269) (8.2, 10.0)	Not Calculable
		Negative	0.6 (1/166) (0.0, 2.8)		0.0 (0/120) (0.0, 2.5)	
		Prevalence (%)	6.5 (27/415)		6.4 (25/389)	
	30 to 39 Years		N=261		N=238	
		Positive	8.2 (8/98) (5.0, 10.1)	13.3 (1.7, 105)	7.1 (7/99) (4.0, 9.2)	4.9 (1.0, 23.2)
		Negative	0.6 (1/163) (0.0, 2.4)		1.4 (2/139) (0.2, 3.6)	
		Prevalence (%)	3.4 (9/261)		3.8 (9/238)	
	≥ 40 Years		N=261		N=236	
		Positive	5.9 (3/51) (1.6, 9.7)	6.2 (1.1, 36.0)	7.5 (4/53) (2.9, 10.7)	13.8 (1.6, 121)
		Negative	1.0 (2/210) (0.1, 2.0)		0.5 (1/183) (0.0, 1.8)	
		Prevalence (%)	1.9 (5/261)		2.1 (5/236)	

*74 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

NILM ≥ 30 Years Population: Aptima HPV Assay Clinical Performance at Baseline

In total, there were 11,644 women with NILM cytology results enrolled in the NILM Study, of these, 773 women were withdrawn. The remaining 10,871 women were eligible for testing on the Panther System. Eleven women had missing samples and were excluded from the baseline evaluation of the Aptima HPV assay on the Panther System. The remaining 10,860 evaluable women were 30 years of age and older with NILM cytology results and Aptima HPV assay results on the Panther System. Of the 512 women with positive Aptima HPV assay results on the Panther System, 284 attended colposcopy at baseline. Of the 10,348 women with negative Aptima HPV assay results, 580 attended colposcopy at baseline. Twenty (20) women had ≥CIN2 and eleven (11) had ≥CIN3; 798 women had Normal/CIN1 histology; 46 women had undetermined disease status. The results of the Aptima HPV assay on the Panther System by the Consensus Histology Review Panel diagnosis at baseline are presented in Table 36.

Table 36: NILM ≥ 30 Years Population: Results of the Aptima HPV Assay and an FDA-approved HPV DNA Test by Consensus Histology Review Panel Diagnosis at Baseline

Aptima HPV Assay Result*	HPV DNA Test	Consensus Histology Review Panel Diagnosis						
		Undetermined**	Normal	CIN1	CIN2	CIN3	Cancer	Total
Positive	Positive	11	211	12	4	7	2	247
Positive	Negative	2	19	0	0	0	1	22
Positive	No Result***	2	12	1	0	0	0	15
Negative	Positive	10	170	7	2	1	0	190
Negative	Negative	20	353	9	2	0	0	384
Negative	No Result***	1	4	0	1	0	0	6
Total		46	769	29	9	8	3****	864

*All samples had final valid results (upon initial testing or after resolution of initial invalids per procedure).
**46 subjects attended the colposcopy visit but a diagnosis could not be determined for the following reasons: biopsy specimens determined to be inadequate (n=29), no biopsies collected (n=15), and biopsy slides lost (n=2).
***21 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.
****Three women had adenocarcinoma in situ (AIS).

In total, 10,042 women had unverified (including undetermined) disease status at baseline (Table 37). Because only randomly selected women with negative results for both the Aptima HPV assay on the Tigris DTS System and the FDA-approved HPV DNA test were referred to colposcopy, the proportion of women with unverified disease status was high in this group (96.6%). To adjust for this verification bias, a multiple imputation method was used to estimate the number of women with disease that would have been identified if all women had undergone colposcopy. Both verification-bias adjusted performance estimates and unadjusted performance estimates based on the 818 women with verified disease status at baseline are presented.

Table 37: NILM ≥ 30 Years Population: Classification of Evaluable NILM Women by Aptima HPV Assay and an FDA-approved HPV DNA Test Results, Disease Status (≥CIN2 and ≥CIN3), and Disease Verification Status

Aptima HPV Assay Result*		HPV DNA Test	Total Women	Verified Disease Status: ≥CIN2		Verified Disease Status: ≥CIN3		Unverified Disease Status
Panther System	Tigris DTS System			Diseased Women (≥CIN2)	Non-Diseased Women (<CIN2)	Diseased Women (≥CIN3)	Non-Diseased Women (<CIN3)	Women with Unknown Disease Status (% Unknown)
Positive	**Positive**	Positive	313	13	189	9	193	111 (35.5%)
Positive	**Positive**	Negative	37	1	18	1	18	18 (48.6%)
Positive	**Positive**	No Result**	22	0	13	0	13	9 (40.9%)
Positive	**Negative**	Positive	70	0	34	0	34	36 (51.4%)
Positive	**Negative**	Negative	60	0	1	0	1	59 (98.3%)
Positive	**Negative**	No Result**	10	0	0	0	0	10 (100%)
Negative	**Positive**	Positive	46	0	33	0	33	13 (28.3%)
Negative	**Positive**	Negative	113	1	41	0	42	71 (62.8%)
Negative	**Positive**	No Result**	8	0	4	0	4	4 (50.0%)
Negative	**Negative**	Positive	236	3	144	1	146	89 (37.7%)
Negative	**Negative**	Negative	9,354	1	321	0	322	9032 (96.6%)
Negative	**Negative**	No Result**	591	1	0	0	1	590 (99.8%)
Total			10,860	20	798	11	807	10,042 (92.5%)

*All samples had final results (upon initial testing or after resolution of initial invalids per procedure).
**631 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

The adjusted prevalence of ≥CIN2 and ≥CIN3 in women with NILM cytology results were 0.9% and 0.4%, respectively. The adjusted absolute and relative risk estimates for detection of ≥CIN2 and ≥CIN3 at baseline are shown in Table 38. The adjusted relative risk of ≥CIN2 was 7.5 (95% CI: 2.1, 26.3), indicating that a woman who was Aptima HPV assay positive is 7.5 times as likely to have ≥CIN2 than a woman who is Aptima HPV assay negative. The adjusted relative risk of ≥CIN3 was 24.9 (95% CI: 2.0, 307.0). The unadjusted absolute and relative risk estimates for detection of ≥CIN2 and ≥CIN3 at baseline are shown overall in Table 39 and by age group in Table 40.

Table 38: NILM ≥ 30 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an FDA-approved HPV DNA Test (Verification-Bias Adjusted Estimates) at Baseline

		Aptima HPV Assay		HPV DNA Test	
	Assay Result	Absolute Risk (95% CI)	Relative Risk (95% CI)	Absolute Risk (95% CI)	Relative Risk (95% CI)
≥CIN2	Positive	4.5 (2.7, 7.4)	7.5 (2.1, 26.3)	3.7 (2.3, 6.1)	7.3 (1.6, 33.5)
	Negative	0.6 (0.2, 1.9)		0.5 (0.1, 2.1)	
	Prevalence (%)	0.9		0.9	
≥CIN3	Positive	3.0 (1.6, 5.5)	24.9 (2.0, 307.0)	2.3 (1.3, 4.1)	21.0 (1.0, 423.8)
	Negative	0.1 (0.0, 1.7)		0.1 (0.0, 2.4)	
	Prevalence (%)	0.4		0.4	

Table 39: NILM ≥ 30 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an FDA-approved HPV DNA Test (Unadjusted Estimates) at Baseline

		Aptima HPV Assay N=818		HPV DNA Test N=800*	
	Assay Result	Absolute Risk (95% CI)	Relative Risk (95% CI)	Absolute Risk (95% CI)	Relative Risk (95% CI)
≥CIN2	Positive	5.2 (14/269) (3.5, 6.6)	4.8 (1.9, 12.3)	3.8 (16/416) (2.9, 4.5)	4.9 (1.4, 16.8)
	Negative	1.1 (6/549) (0.5, 1.9)		0.8 (3/384) (0.2, 1.9)	
	Prevalence (%)	2.4 (20/818)		2.4 (19/800)	
≥CIN3	Positive	3.7 (10/269) (2.5, 4.3)	20.4 (2.6, 159)	2.4 (10/416) (1.6, 2.7)	9.2 (1.2, 71.8)
	Negative	0.2 (1/549) (0.0, 0.8)		0.3 (1/384) (0.0, 1.1)	
	Prevalence (%)	1.3 (11/818)		1.4 (11/800)	

*18 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

Table 40: NILM ≥ 30 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an FDA-approved HPV DNA Test by Age Group (Unadjusted Estimates) at Baseline

	Age	Assay Result	Aptima HPV Assay N=818		HPV DNA Test N=800*	
			Absolute Risk (95% CI)	Relative Risk (95% CI)	Absolute Risk (95% CI)	Relative Risk (95% CI)
≥CIN2	30 to 39 Years		N=383		N=376	
		Positive	4.6 (7/153) (2.5, 5.9)	5.3 (1.1, 25.0)	3.3 (7/215) (1.8, 4.1)	2.6 (0.6, 12.4)
		Negative	0.9 (2/230) (0.1, 2.2)		1.2 (2/161) (0.2, 3.2)	
		Prevalence (%)	2.3 (9/383)		2.4 (9/376)	
	≥ 40 Years		N=435		N=424	
		Positive	6.0 (7/116) (3.2, 8.5)	4.8 (1.4, 16.1)	4.5 (9/201) (2.9, 5.3)	10.0 (1.3, 78.1)
		Negative	1.3 (4/319) (0.4, 2.3)		0.4 (1/223) (0.0, 1.8)	
		Prevalence (%)	2.5 (11/435)		2.4 (10/424)	
≥CIN3	30 to 39 Years		N=383		N=376	
		Positive	3.3 (5/153) (1.6, 4.1)	7.5 (0.9, 63.7)	2.3 (5/215) (1.1, 2.9)	3.7 (0.4, 31.7)
		Negative	0.4 (1/230) (0.0, 1.6)		0.6 (1/161) (0.0, 2.2)	
		Prevalence (%)	1.6 (6/383)		1.6 (6/376)	
	≥ 40 Years		N=435		N=424	
		Positive	4.3 (5/116) (2.2, 5.1)	Not Calculable	2.5 (5/201) (1.3, 2.8)	Not Calculable
		Negative	0.0 (0/319) (0.0, 0.8)		0.0 (0/223) (0.0, 1.1)	
		Prevalence (%)	1.1 (5/435)		1.2 (5/424)	

*18 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

Adjusted clinical performance estimates of the Aptima HPV assay including sensitivity, specificity, PPV, and NPV for the detection of ≥CIN2 and ≥CIN3 at baseline are shown in Table 41, as are the estimates for the FDA-approved HPV DNA test. Unadjusted clinical performance estimates are shown in Table 42. The Aptima HPV assay and the FDA-approved HPV DNA test had similar sensitivity, whereas specificity was significantly higher for the Aptima HPV assay (non-overlapping 95% CIs). Predictive value estimates of the Aptima HPV assay were clinically relevant and similar to the estimates for the FDA-approved HPV DNA test. NPVs were similar but for the detection of ≥CIN2, the PPV for the Aptima HPV assay was slightly higher than PPV for the FDA-approved HPV DNA test (4.5% vs 3.7%).

Table 41: NILM ≥ 30 Years Population: Performance of the Aptima HPV Assay and an FDA-approved HPV DNA Test for Detection of ≥CIN2 and ≥CIN3 (Verification-Bias Adjusted Estimates) at Baseline

		Aptima HPV Assay		HPV DNA Test	
	Performance	Estimate	(95% CI)	Estimate	(95% CI)
≥CIN2	Sensitivity (%)	28.4	(4.9, 51.8)	35.4	(3.8, 66.9)
	Specificity (%)	95.5	(95.1, 95.9)	93.7	(93.2, 94.2)
	PPV (%)	4.5	(2.7, 7.4)	3.7	(2.3, 6.1)
	NPV (%)	99.4	(98.1, 99.8)	99.5	(97.9, 99.9)
	Prevalence (%)	0.9 (0.0, 1.9)		0.9 (0.0, 1.9)	
≥CIN3	Sensitivity (%)	54.0	(3.6, 100)	56.4	(0.4, 100)
	Specificity (%)	95.4	(95.0, 95.8)	93.6	(93.1, 94.1)
	PPV (%)	3.0	(1.6, 5.5)	2.3	(1.3, 4.1)
	NPV (%)	99.9	(98.3, 100)	99.9	(97.6, 100)
	Prevalence (%)	0.4 (0.0, 1.2)		0.4 (0.0, 1.3)	

Table 42: NILM ≥ 30 Years Population: Performance of the Aptima HPV Assay and an FDA-approved HPV DNA Test for Detection of ≥CIN2 and ≥CIN3 (Unadjusted Estimates) at Baseline

		Aptima HPV Assay N=818		HPV DNA Test N=800*	
	Performance	**Estimate**	**(95% CI)**	**Estimate**	**(95% CI)**
≥CIN2	**Sensitivity (%)**	70.0 (14/20)	(48.1, 85.5)	84.2 (16/19)	(62.4, 94.5)
	Specificity (%)	68.0 (543/798)	(64.7, 71.2)	48.8 (381/781)	(45.3, 52.3)
	PPV (%)	5.2 (14/269)	(3.5, 6.6)	3.8 (16/416)	(2.9, 4.5)
	NPV (%)	98.9 (543/549)	(98.1, 99.5)	99.2 (381/384)	(98.1, 99.8)
	Prevalence (%)	2.4 (20/818)		2.4 (19/800)	
≥CIN3	**Sensitivity (%)**	90.9 (10/11)	(62.3, 98.4)	90.9 (10/11)	(62.3, 98.4)
	Specificity (%)	67.9 (548/807)	(64.6, 71.0)	48.5 (383/789)	(45.1, 52.0)
	PPV (%)	3.7 (10/269)	(2.5, 4.3)	2.4 (10/416)	(1.6, 2.7)
	NPV (%)	99.8 (548/549)	(99.2, 100)	99.7 (383/384)	(98.9, 100)
	Prevalence (%)	1.3 (11/818)		1.4 (11/800)	

*18 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

Direct comparison of the Aptima HPV assay on the Panther System and the FDA-approved HPV DNA test demonstrates similar sensitivity and statistically significant improved specificity of the Aptima HPV assay over the FDA-approved HPV DNA test for detection of ≥CIN2 as shown by the ratios of true positive and false positive rates (Table 43 and Table 44, respectively).

Table 43: NILM ≥ 30 Years Population: Ratio of True Positive Rates (Aptima HPV Assay/ FDA-approved HPV DNA Test) for Women with ≥CIN2 (Unadjusted Estimates) at Baseline

		HPV DNA Test		
		Positive	Negative	Total
Aptima HPV Assay	Positive	13	1	14 (73.7%)
	Negative	3	2	5
	Total	16 (84.2%)	3	19
Ratio of True Positive Rates = 0.88 (14/16) (95% CI: 0.65, 1.10)				

Table 44: NILM ≥ 30 Years Population: Ratio of False Positive Rates (Aptima HPV Assay/ FDA-approved HPV DNA Test) for Women with <CIN2 (Unadjusted Estimates) at Baseline

		HPV DNA Test		
		Positive	Negative	Total
Aptima HPV Assay	Positive	223	19	242 (31.0%)
	Negative	177	362	539
	Total	400 (51.2%)	381	781
Ratio of False Positive Rates = 0.61 (242/400) (95% CI: 0.55, 0.66)				

NILM ≥ 30 Years Population: Aptima HPV Assay on the Panther System Clinical Performance After 3 Years of Follow-up

The were 10,843 women 30 years of age and older with NILM cytology results and valid 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% (7,247/10,823) of women completed a year 1 follow-up Pap visit, 60.3% (6,517/10,814) the year 2 and 58.7% (6,339/10,807) the year 3. Overall, 58.8% (6,375/10,843) of the women completed the study (had ≥CIN2 at baseline or during follow-up, and/or completed required visits).

Of the 10,843 evaluable women, 511 (4.7%) had positive Aptima HPV assay results on the Panther system at baseline. Of these 511 women, 255 (49.9%) had either positive or negative 3-year disease status based on cytology or colposcopy/biopsy results. The remaining 10,332 women had negative Aptima HPV assay results on the Panther system at baseline. Of these 10,332 women, 5,946 (57.5%) had either positive or negative 3-year disease status. Of the 6,201 women with 3-year disease status, 47 women had ≥CIN2 including 23 with ≥CIN3; 6,154 women had normal/CIN1 by Consensus Histology Review Panel. The baseline results of the Aptima HPV assay on the Panther system and FDA-approved HPV DNA assay, and the 3-year disease status (includes baseline and follow-up evaluation) by Consensus Histology Review Panel are presented in Table 45.

Table 45: NILM ≥ 30 Years Population: Classification of Women Eligible for the Follow-up Phase by Baseline Aptima HPV Assay, Baseline FDA-Approved HPV DNA Test Results, and Disease Status (≥CIN2, ≥CIN3, Unverified) Determined in the Baseline and Follow-up Phases

Aptima HPV 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)	Lost to Follow-up	Indeterminate*
Positive	Positive	382	23	171	16	178	167	21
Positive	Negative	97	1	48	1	48	44	4
Positive	No Result**	32	2	10	1	11	17	3
Negative	Positive	281	5	129	2	132	130	17
Negative	Negative	9,452	15	5,476	3	5,488	3,756	205
Negative	No Result**	599	1	320	0	321	264	14
	Total	10,843	47	6,154	23	6,178	4,378	264

*Women who had abnormal cytology test results during follow-up and who did not have a subsequent Consensus Histology Review Panel result, and women with inadequate cytology at their last visit. 174 women with indeterminate disease status completed their follow-up per protocol.
**631 women with Aptima HPV assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

The 3-year cumulative risk 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 and relative risk estimates for detection of ≥CIN2 and ≥CIN3 are shown in Table 46.

Table 46: NILM ≥ 30 Years Population: 3-Year Cumulative Absolute and Relative Risks* of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an FDA-approved HPV DNA Test at Baseline

		Aptima HPV Assay		HPV DNA Test	
	Assay Result	Absolute Risk (95% CI)	Relative Risk (95% CI)	Absolute Risk (95% CI)	Relative Risk (95% CI)
≥CIN2	Positive	7.90 (5.50, 11.27)	24.45 (13.85, 43.15)	6.43 (4.50, 9.14)	22.71 (12.20, 42.30)
	Negative	0.32 (0.21, 0.51)		0.28 (0.17, 0.47)	
	Prevalence (%)	0.68		0.68	
≥CIN3	Positive	5.23 (3.34, 8.13)	57.11 (21.09, 154.62)	4.14 (2.62, 6.52)	51.34 (17.74, 148.58)
	Negative	0.09 (0.04, 0.23)		0.08 (0.03, 0.22)	
	Prevalence (%)	0.34		0.35	

*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.

The 3-year cumulative prevalence of ≥CIN2 and ≥CIN3 in women with NILM cytology results at baseline were 0.68% and 0.34%, respectively. The relative risk of ≥CIN2 was 24.45 (95% CII 13.85, 43.15), indicating that a woman who was Aptima HPV assay positive on the Panther system is 24.45 times more likely to have ≥CIN2 than a woman who is Aptima HPV assay negative. The relative risk of ≥CIN3 was 57.11 (95% CI: 21.09, 154.62).

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

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

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

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

The agreement of the Aptima HPV assay results assessed in Pre-cytology and Post-cytology samples is shown in Table 47.

Table 47: Agreement Results of the Aptima HPV Assay in Pre- and Post-cytology Samples

Category	Agreement	A vs B	A vs C	B vs A	B vs C
Total population[1]	**PPA % (95% CI)[2] n/N**	89.2 (84.5, 92.6) 206/231	87.4 (82.6, 91.1) 202/231	85.8 (80.9, 89.7) 206/240	85.8 (80.9, 89.7) 206/240
	NPA % (95% CI)[2] n/N	98.0 (97.2, 98.6) 1677/1711	97.6 (96.8, 98.2) 1670/1711	98.5 (97.8, 99.0) 1677/1702	97.8 (97.0, 98.4) 1665/1702
NILM (≥30 years)	**PPA % (95% CI)[2] n/N**	76.5 (67.2, 83.8) 75/98	74.5 (65.0, 82.1) 73/98	72.1 (62.8, 79.8) 75/104	73.1 (63.8, 80.7) 76/104
	NPA % (95% CI)[2] n/N	98.2 (97.5, 98.8) 1605/1634	97.8 (97.0, 98.4) 1598/1634	98.6 (97.9, 99.1) 1605/1628	98.0 (97.2, 98.6) 1595/1628
ASC-US (≥21 years)	**PPA % (95% CI)[2] n/N**	98.2 (90.7, 99.7) 56/57	96.5 (88.1, 99.0) 55/57	94.9 (86.1, 98.3) 56/59	93.2 (83.8, 97.3) 55/59
	NPA % (95% CI)[2] n/N	95.4 (87.3, 98.4) 62/65	98.5 (91.8, 99.7) 64/65	98.4 (91.5, 99.7) 62/63	98.4 (91.5, 99.7) 62/63

CI = confidence interval, NPA = negative percent agreement, PPA = positive percent agreement.
[1] Total population includes >ASC-US, NILM, ASC-US.
[2] Score CI

Table 48 shows the agreement based on samples where pre-cytology A and B samples gave the same result (ie, both positive or both negative).

Table 48: Agreement Results of the Aptima HPV Assay in Pre- and Post-cytology Samples When Pre-cytology Sample Results Agree

Category	PPA % (95% CI)[1] n/N	NPA % (95% CI)[1] n/N	Discordant	
Total population[2]	94.2 (90.1, 96.6) 194/206	98.3 (97.5, 98.8) 1648/1677	A-B+	34
			A+B-	25
NILM (≥30 years)	89.3 (80.3, 94.5) 67/75	98.3 (97.6, 98.8) 1578/1605	A-B+	29
			A+B-	23
ASC-US (≥21 years)	96.4 (87.9, 99.0) 54/56	100 (94.2, 100) 62/62	A-B+	3
			A+B-	1

CI = confidence interval, NPA = negative percent agreement, PPA = positive percent agreement.
[1] Score CI
[2] Total population includes >ASC-US, NILM, ASC-US.

Aptima HPV Assay Agreement with a Composite Comparator

The analytical performance of the Aptima HPV assay on the Panther System was assessed against a composite comparator consisting of an FDA-approved HPV DNA test and a 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 were tested by the comparator assays and compared to the Aptima HPV assay results.[17] In total, 434 samples were tested, with 217 from each of the ASC-US and NILM populations. Women selected included approximately 150 randomly selected women with positive Aptima HPV assay results on the Tigris DTS System and <CIN2 disease, approximately 200 randomly selected women with negative Aptima HPV assay results on the Tigris DTS System and <CIN2 disease and all women identified with ≥CIN2 disease by consensus histology review at the time of sample selection (when about 80% of the trial enrollment was completed).

For the composite comparator analysis, samples were classified positive if the HPV DNA test and the E6/E7 RT-PCR sequencing test were both positive; negative if the HPV DNA test and the E6/E7 RT-PCR sequencing test were both negative; and indeterminate if the tests were discordant, or if one or both tests returned an invalid or indeterminate result.

Positive and negative percent agreements and associated 95% confidence intervals were calculated. Indeterminate results were not included in the agreement calculations. Results are presented for the ASC-US population (≥ 21 years) and the NILM population (≥ 30 years) in Table 49a and Table 49b, respectively.

Table 49a: ASC-US ≥ 21 Years Population: Aptima HPV Assay Agreement Results with a Composite Comparator (n=217)

		Composite Comparator			
		Positive	**Negative**	**Indeterminate**	**Total**
Aptima HPV Assay	**Positive**	88	1	29	118
	Negative	3	85	11	99
	Total	91	86	40	217
Positive Percent Agreement: 96.7% (88/91) (95% CI: 90.8, 98.9) **Negative Percent Agreement:** 98.8% (85/86) (95% CI: 93.7, 99.8)					

Table 49b: NILM ≥ 30 Years Population: Aptima HPV Assay Agreement Results with a Composite Comparator (n=217)

		Composite Comparator			
		Positive	**Negative**	**Indeterminate**	**Total**
Aptima HPV Assay	**Positive**	54	4	40	98
	Negative	5	74	40	119
	Total	59	78	80	217

Table 49b: NILM ≥ 30 Years Population: Aptima HPV Assay Agreement Results with a Composite Comparator (n=217)

	Composite Comparator			
	Positive	Negative	Indeterminate	Total
Positive Percent Agreement: 91.5% (54/59) (95% CI: 81.6, 96.3)				
Negative Percent Agreement: 94.9% (74/78) (95% CI: 87.5, 98.0)				

Clinical Cutoff Determination for the Aptima HPV Assay

The method used to establish the clinical cutoff for detecting high-grade cervical disease (≥CIN2) for the Aptima HPV assay is described in *Clinical Cutoff Determination for the Aptima HPV Assay* in the Tigris DTS System section. The cutoff for the Aptima HPV assay was set at 0.50 S/CO.

Limit of Detection at the Clinical Cutoff

The Limit of Detection (LOD) at the clinical cutoff is the concentration of HPV RNA that gives a positive result (above the clinical cutoff) 95% of the time. The LOD of the Aptima HPV assay was determined by testing dilution panels of in vitro transcripts (IVT) for all 14 high-risk genotypes and 4 HPV-infected cell lines: SiHa, HeLa, MS751 and ME180 (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 two reagent lots for a total of 60 replicates. Testing was performed over 17 days, with 1 to 12 runs performed per day and 5 replicates of a given genotype and concentration tested in each run. The 95% detection limit was calculated from Probit regression analysis of the positivity results for each dilution panel.

The Probit analysis results in Table 50 show that HPV 16, 18, 31, 33, 35, 39, 45, 51, 56, 59, and 68 had 95% detection limits less than 100 copies/reaction; and types 52, 58, and 66 had 95% detection limits between 100 and 500 copies/reaction. The four cell lines tested had 95% detection limits less than 1 cell/reaction.

Table 50: Limit of Detection at Clinical Cutoff of the Aptima HPV Assay

Target	Limit of Detection* (95% CI)
HPV 16	49.4 (37.1 - 73.0)
HPV 18	44.0 (34.4 - 62.1)
HPV 31	32.5 (23.2 - 52.1)
HPV 33	67.5 (48.8 - 106.2)
HPV 35	32.7 (23.6 - 51.4)
HPV 39	20.9 (16.3 - 29.5)
HPV 45	37.1 (27.9 - 54.7)
HPV 51	51.1 (36.3 - 83.9)
HPV 52	410.2 (310.7 - 595.1)
HPV 56	59.4 (46.7 - 81.5)
HPV 58	124.1 (90.7 - 190.1)
HPV 59	81.1 (61.9 - 116.6)
HPV 66	118.5 (83.2 - 202.0)
HPV 68	22.4 (17.1 - 32.4)
SiHa	0.25 (0.19 - 0.36)
HeLa	0.11 (0.09 - 0.14)
ME180	0.10 (0.08 - 0.16)
MS751	0.17 (0.14 - 0.25)

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

Assay Precision

Aptima HPV assay precision was evaluated in two studies using the same 20-member panel. Study 1 was conducted at 3 sites, 2 external and 1 internal, and Study 2 was conducted in-house. The panel included 13 HPV-positive members with concentrations at or above the limit of detection of the assay (expected positivity: ≥ 95%), 3 HPV-positive members with concentrations below the limit of detection of the assay (expected positivity: >0% to <25%), and 4 HPV-negative members. HPV-positive panel members were prepared by spiking in vitro RNA transcripts (IVT) into PreservCyt Solution diluted with specimen transport medium (STM) or HPV-infected cultured cells (SiHa, HeLa, and MS751; ATCC, Manassas, Virginia) into pooled negative ThinPrep liquid cytology specimens diluted with STM. HPV-negative panel members were prepared with PreservCyt Solution or pooled negative ThinPrep liquid cytology specimens diluted with STM.

In Study 1, 2 operators at each of the 3 testing sites (1 instrument per site) performed 2 Aptima HPV assay worklists per day (1 with each reagent lot) over 3 days. 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 worklists 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 51a (panel members with expected positive results) and Table 51b (panel members with expected negative results), along with a summary of the agreement with expected results and analyte S/CO values at the 2.5th, 50th and 97.5th percentiles of the S/CO distribution. The analyte S/CO variability for the panel members with expected positive results is shown in Table 52 for Study 1 and Table 53 for Study 2.

Table 51a: Aptima HPV Assay Precision Study 1 and 2: Panel Description, Positive Agreement, and Percentile Distribution of Analyte S/CO Values for Panel Members with Expected Positive Results

Panel Description (copies or cells/reaction)	Study 1 (3 testing sites)				Study 2 (1 testing site)			
	% positive agreement (95% CI)	Analyte S/CO Percentile			% positive agreement (95% CI)	Analyte S/CO Percentile		
		2.5th	50th	97.5th		2.5th	50th	97.5th
HPV high positive clinical sample 1	100 (107/107) (96.5, 100)	21.16	29.64	33.63	100 (161/161) (97.7, 100)	22.50	26.84	30.67
HPV high positive clinical sample 2	100 (107/107) (96.5, 100)	25.98	29.77	36.03	100 (162/162) (97.7, 100)	25.00	28.61	33.99
HPV 16 IVT (1830 copies)	100 (107/107) (96.5, 100)	10.45	11.18	12.40	100 (161/161) (97.1, 100)	10.40	11.07	11.75
HPV 18 IVT (1550 copies)	100 (107/107) (96.5, 100)	13.09	14.55	18.08	100 (162/162) (97.7, 100)	11.26	13.47	15.63
HPV low positive clinical sample 1	94.4 (101/107) (88.3, 97.4)	0.00	9.93	11.03	89.5 (145/162) (83.3, 93.3)	0.00	9.53	10.95
HPV low positive clinical sample 2	88.0 (95/108) (80.5, 92.8)	0.00	7.30	16.63	92.0 (149/162) (86.8, 95.3)	0.00	7.56	19.67
HPV low positive clinical sample 3	100 (108/108) (96.6, 100)	2.80	10.19	17.08	97.5 (157/161) (93.8, 99.0)	1.14	9.53	15.38
HPV low positive clinical sample 4	90.7 (98/108) (83.8, 94.9)	0.00	4.48	11.16	92.6 (150/162) (87.5, 95.7)	0.00	4.66	12.00
HPV 16 IVT (183 copies)	100 (102/102) (96.4, 100)	10.03	11.14	11.97	100 (162/162) (97.7, 100)	10.24	11.05	11.85
HPV 18 IVT (155 copies)	100 (108/108) (96.6, 100)	4.87	12.01	15.21	100 (159/159) (97.6, 100)	7.82	11.59	13.84
MS751 cells (0.63 cells)	100 (108/108) (96.6, 100)	5.90	10.99	14.00	100 (162/162) (97.7, 100)	5.61	10.14	12.26
HeLa cells (0.35 cells)	100 (108/108) (96.6, 100)	1.43	6.19	13.28	100 (162/162) (97.7, 100)	3.24	7.88	12.58
SiHa cells (0.90 cells)	87.9 (94/107) (80.3, 92.8)	0.00	9.80	11.04	89.5 (145/162) (83.8, 93.3)	0.00	9.19	10.94

IVT = in vitro transcript

*Expected % positive agreement ~95%; observed lower possibly due to manufacturing variability of the panel member.

Table 51b: Aptima HPV Assay Precision Study 1 and 2: Panel Description, Negative Agreement, and Percentile Distribution of Analyte S/CO Values for Panel Members with Expected Negative Results

Panel Description (copies or cells/reaction)	Study 1 (3 testing sites)				Study 2 (1 testing site)			
	% negative agreement (95% CI)	Analyte S/CO Percentile			% negative agreement (95% CI)	Analyte S/CO Percentile		
		2.5th	50th	97.5th		2.5th	50th	97.5th
MS751 cells (0.005 cells)	87.0 (94/108) (79.4, 92.1)	0.00	0.00	4.37	93.8 (152/162) (89.0, 96.6)	0.00	0.00	2.25
SiHa cells (0.008 cells)	97.2 (105/108) (92.1, 99.1)	0.00	0.00	1.53	95.7 (155/162) (91.4, 97.9)	0.00	0.00	7.56
HeLa cells (0.02 cells)	70.4 (76/108) (61.2, 78.2)	0.00	0.00	3.95	67.3 (109/162) (59.8, 74.0)	0.00	0.12	6.35
HPV-negative clinical sample 1	99.1 (107/108) (94.9, 99.8)	0.00	0.00	0.33	100 (162/162) (97.7, 100)	0.00	0.00	0.07
HPV-negative clinical sample 2	97.2 (105/108) (92.1, 99.1)	0.00	0.00	1.21	100 (162/162) (97.7, 100)	0.00	0.00	0.05
PreservCyt Solution 1	99.1 (107/108) (94.9, 99.8)	0.00	0.00	0.15	100 (162/162) (97.7, 100)	0.00	0.00	0.06
PreservCyt Solution 2	99.1 (107/108) (94.9, 99.8)	0.00	0.00	0.22	100 (161/161) (97.7, 100)	0.00	0.00	0.09

IVT = in vitro transcript.

*Expected % negative agreement > 75% and < 100%.

Table 52: Aptima HPV Assay Precision Study 1: Signal Variability for Panel Members With Expected Positive Results

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 high positive clinical sample 1	107*	29.34	0.00	0.0	0.00	0.0	1.43	4.9	1.87	6.4	1.49	5.1	2.79	9.5
HPV high positive clinical sample 2	107*	30.09	0.55	1.8	0.00	0.0	1.06	3.5	0.73	2.4	2.21	7.3	2.61	8.7
HPV 16 IVT (1830 copies)	107*	11.20	0.09	0.8	0.16	1.4	0.03	0.3	0.14	1.3	0.46	4.1	0.52	4.6
HPV 18 IVT (1550 copies)	107*	14.89	0.18	1.2	0.00	0.0	0.20	1.3	0.14	0.9	1.53	10.3	1.56	10.5
HPV low positive clinical sample 1	107*	8.24	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	3.23	39.2	3.23	39.2
HPV low positive clinical sample 2	108	7.07	0.00	0.0	0.41	5.8	0.00	0.0	0.00	0.0	4.57	64.7	4.59	65.0
HPV low positive clinical sample 3	108	10.23	0.26	2.5	0.00	0.0	0.00	0.0	1.32	12.9	3.23	31.6	3.49	34.2
HPV low positive clinical sample 4	108	4.68	0.50	10.7	0.20	4.2	0.00	0.0	0.99	21.1	3.02	64.6	3.22	68.9
HPV 16 IVT (183 copies)	102*	11.09	0.08	0.7	0.00	0.0	0.00	0.0	0.26	2.3	0.54	4.9	0.61	5.5
HPV 18 IVT (155 copies)	108	11.78	0.00	0.0	0.43	3.7	0.00	0.0	1.12	9.5	1.97	16.7	2.30	19.6
MS751 cells (0.63 cells)	108	10.73	0.00	0.0	0.59	5.5	0.72	6.7	0.82	7.6	1.86	17.3	2.23	20.8
HeLa cells (0.35 cells)	108	6.78	0.00	0.0	0.56	8.3	0.00	0.0	1.23	18.2	3.08	45.5	3.37	49.7
SiHa cells (0.90 cells)	107*	7.74	0.37	4.8	0.00	0.0	0.00	0.0	0.00	0.0	3.85	49.8	3.87	50.1

*Twelve samples had invalid Aptima HPV assay results (1 for HPV high positive clinical sample 1, 1 for HPV high positive clinical sample 2, 1 for HPV 16 IVT (1830 copies), 1 for HPV 18 IVT (1550 copies), 1 for HPV low positive clinical sample 1, 6 for HPV 16 IVT (183 copies), and 1 for SiHa cells (0.90 cells)).

CV = coefficient of variation; IVT = in vitro transcript; 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 53: Aptima HPV Assay Precision Study 2: Signal Variability for Panel Members with Expected Positive Results

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 high positive clinical sample 1	161*	26.81	0.75	2.8	0.00	0.0	0.91	3.4	0.48	1.8	1.84	6.9	2.24	8.3
HPV high positive clinical sample 2	162	28.83	0.00	0.0	0.00	0.0	0.96	3.3	0.65	2.3	2.35	8.2	2.62	9.1
HPV 16 IVT (1830 copies)	161*	11.07	0.14	1.2	0.00	0.0	0.05	0.5	0.16	1.4	0.32	2.9	0.39	3.5
HPV 18 IVT (1550 copies)	162	13.34	0.14	1.1	0.12	0.9	1.00	7.5	0.31	2.3	0.75	5.6	1.31	9.8
HPV low positive clinical sample 1	162	7.57	0.56	7.5	0.55	7.3	0.63	8.3	0.00	0.0	3.61	47.7	3.75	49.5
HPV low positive clinical sample 2	162	7.59	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	5.25	69.2	5.25	69.2
HPV low positive clinical sample 3	161*	8.83	0.00	0.0	0.00	0.0	0.26	3.0	0.00	0.0	3.48	39.4	3.49	39.5
HPV low positive clinical sample 4	162	4.95	0.00	0.0	0.00	0.0	0.75	15.2	0.00	0.0	3.35	67.6	3.43	69.3
HPV 16 IVT (183 copies)	162	11.02	0.13	1.2	0.11	1.0	0.12	1.1	0.13	1.2	0.54	4.9	0.59	5.4
HPV 18 IVT (155 copies)	159*	11.40	0.16	1.4	0.17	1.5	1.21	10.6	0.23	2.0	1.17	10.3	1.72	15.0
MS751 cells (0.63 cells)	162	9.87	0.76	7.7	0.00	0.0	0.65	6.6	0.65	6.6	1.41	14.3	1.85	18.7
HeLa cells (0.35 cells)	162	7.80	0.55	7.0	0.00	0.0	0.85	10.9	0.00	0.0	2.44	31.3	2.65	33.9
SiHa cells (0.90 cells)	162	7.30	0.32	4.3	0.00	0.0	0.93	12.7	1.04	14.3	3.49	47.8	3.77	51.7

*Six samples had invalid Aptima HPV assay results (1 for HPV high positive clinical samples 1, 1 for HPV 16 IVT (1830 copies), 1 for HPV low positive clinical sample 3, 3 for HPV 18 IVT (155 copies).

CV = coefficient of variation; IVT = in vitro transcript; 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

Testing with potentially cross-reactive organisms for the Aptima HPV assay was performed using the Tigris DTS System. Refer to *Cross-Reactivity* (Table 26) in the Tigris DTS System section for results.

Interference

Testing with potential interfering substances for the Aptima HPV assay was performed using the Tigris DTS System. Refer to *Interference* (Table 27) in the Tigris DTS System section for results.

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