Aptima HPV Assay

For *in vitro* diagnostic use.

For U.S. export only.

General Information

Intended Use

The Aptima HPV assay is a target amplification nucleic acid probe test for the *in vitro* qualitative detection of E6/E7 viral messenger RNA (mRNA) from 14 high-risk types of human papillomavirus (HPV) (16/18/31/33/35/39/45/51/52/56/58/59/66/68). The Aptima HPV assay does not discriminate between the 14 high-risk types.

- The Aptima HPV assay is indicated for use in screening patients with ASC-US (atypical squamous cells of undetermined significance) Pap test results to determine the need for referral to colposcopy. The results of this test are not intended to prevent women from proceeding to colposcopy.
- The Aptima HPV assay can be used with cervical cytology to adjunctively screen (co-testing) 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.
- The Aptima HPV assay can be used as a first-line primary screening test, with or without cervical cytology, to identify women at increased risk for the development of cervical cancer or presence of high-grade disease. This information, together with the physician's assessment of the patient's screening history, other risk factors, and professional guidelines, may be used to guide patient management.

Cervical specimens collected in ThinPrep™ Pap Test vials containing PreservCyt™ Solution may be tested with the Aptima HPV assay either pre- or post-Pap processing, as well as cervical specimens collected with the Aptima Cervical Specimen Collection and Transport Kit. The assay can be used to test these specimen types with either the Direct Tube Sampling (DTS) Systems, the Tigris DTS System, or the Panther System. Cervical specimens collected in SurePath Preservative Fluid may be tested with the Aptima HPV assay on the Tigris DTS System and the Panther System.

Summary and Explanation of the Test

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

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

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

HPV infections are very common and most women will clear HPV infections within 6 to 12 months.[8, 10] 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.[3]

Aptima HPV Assay Clinical Performance in Primary Screening for Cervical Cancer

The clinical performance of the Aptima HPV assay when used in a primary screening modality has been investigated in multiple studies by independent investigators. Thirteen peer-reviewed publications[11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23] from ten separate clinical studies report the performance of Aptima HPV in primary screening in women enrolled in nine countries (China, Canada, France, Mexico, England, Denmark, The Netherlands, The United States, and Germany). The data from these studies show that Aptima HPV has similar clinical performance compared to other clinically validated HPV tests when used for primary screening for cervical pre-cancer and cancer.

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),[24] and detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA).[25] The assay incorporates an Internal Control (IC) to monitor nucleic acid capture, amplification, and detection, as well as operator or instrument error.

Specimens are collected in or transferred to a tube containing specimen transport media (STM) that lyses the cells, releases the mRNA, and protects it from degradation during storage. When the Aptima HPV 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 supernatent 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.[26] 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 a method used to differentiate between the signals from the flasher and glower labels.[26]

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. For additional specific warnings and precautions refer to the DTS Systems, Tigris DTS System, and Panther System Operator's Manuals.

Laboratory Related

C. Use only supplied or specified disposable laboratory ware.

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

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

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

DTS Systems Specific

G. To help prevent lab areas from becoming contaminated with amplicon, the laboratory area should be arranged with a unidirectional workflow from reagent preparation through detection. Specimens, equipment, and reagents should not be returned to the area where a previous step was performed. Also, personnel should not move back into previous work areas without proper contamination safeguards. A separate area for detection is strongly recommended.

Specimen Related

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

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

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

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

L. Upon piercing, liquid can discharge from tube caps under certain conditions. Refer to *DTS Systems Test Procedure*, *Tigris DTS System Test Procedure*, or *Panther DTS System Test Procedure* for more information.

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

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

Assay Related

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

P. Avoid microbial and ribonuclease contamination of reagents.

Q. Do not use kit after its expiration date.

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

S. Aptima Assay Fluids, Aptima Auto Detect Reagents, Aptima System Fluid Preservative (DTS Systems and Tigris DTS System only) and Aptima HPV assay Controls (DTS System and 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.

DTS Systems Specific

V. A minimum of two repeat pipettors must be dedicated for use with this assay: one for use in the **Target Capture** and **Amplification** steps, and one for use in the **Post-Amplification** steps.

W. When using repeat pipettors for reagent addition, do not touch the tube with the pipette tip to prevent carryover from one tube to another.

X. All pipettors must be cleaned regularly as described in *Procedural Notes*.

Y. At least two separate SB100™ instruments are required, one for Target Capture/ Amplification and one for Post-Amplification.

Z. DO NOT reuse sealing cards. New sealing cards should be used for each step.

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 (DTS Systems and 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 15°C to 30°C. Do not refrigerate.

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

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

G. The Aptima HPV assay reagents are stable for a cumulative 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

ThinPrep liquid cytology specimens

1. Collect cervical specimens in ThinPrep Pap Test vials containing PreservCyt Solution with broom-type or cytobrush/spatula collection devices according to the manufacturer's instructions.
2. Prior to or after processing with the ThinPrep 2000 System, ThinPrep 3000 System, ThinPrep 5000 Processor, or 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.

SurePath liquid cytology specimens (Tigris DTS System and Panther System only)

1. Collect a SurePath liquid cytology specimen according to the SurePath Pap Test and/or PrepStain System instructions for use.
2. Transfer the SurePath liquid cytology specimen into an Aptima Specimen Transfer tube according to the instructions in the Aptima Specimen Transfer Kit package insert.

Aptima Cervical Specimen Collection and Transport Kit specimens

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

B. Transport and storage before testing

ThinPrep liquid cytology specimens

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

SurePath liquid cytology specimens (Tigris DTS System and Panther System only)

1. Transport the SurePath liquid cytology specimens at 2°C to 25°C.
2. Specimens should be transferred to an Aptima Specimen Transfer tube within 7 days of collection.
3. Prior to transfer, SurePath liquid cytology specimens should be stored at 2°C to 25°C.
4. SurePath liquid cytology specimens transferred to an Aptima Specimen Transfer tube may be stored at 2°C to 25°C for up to 7 days.

Aptima Cervical Specimen Collection and Transport Kit specimens

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

C. SurePath Liquid Cytology Specimen Treatment (Tigris DTS System and Panther System only)

Note: *SurePath liquid cytology specimens must be treated with the Aptima Transfer Solution prior to testing with the Aptima HPV assay.*

1. Aptima Transfer Solution (Tigris DTS System and Panther System only)

   Treated samples may be stored at 2°C to 8°C for up to 17 days prior to testing with the Aptima HPV assay. Refer to the Aptima Specimen Transfer kit package insert for further details.

D. 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 samples need to be frozen or shipped, remove penetrable cap and place new non-penetrable caps on the specimen tubes. If specimens need to be shipped for testing at another facility, specified temperatures must be maintained. Prior to uncapping previously tested and recapped samples, specimen 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 national and international transportation regulations.*

DTS Systems

Reagents for the Aptima HPV assay are listed below for the DTS Systems. Reagent Identification Symbols are also listed next to the reagent name.

Reagents and Materials Provided

Note: For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologic.com/sds.

Aptima HPV Assay Kit, 100 tests, Cat No. 302610 (4 boxes)

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

Aptima HPV 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 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
	Sealing Cards	1 package
	Reconstitution Collars	3

Aptima HPV Calibrators Box (Cat. No. 302554)
(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 Controls Box (Cat. No. 302556)
(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.
Leader™ HC+ Luminometer	104747
Hologic Target Capture System (TCS)	104555
2 SB100 Dry Heat Bath/Vortexers	105524F
Aptima Auto Detect Kit	301048C
Aptima Assay Fluids Kit	302002C
Micropipettor, 1000 µL RAININ PR1000	104216
2 eppendorf Repeater Plus Pipettors	105725
Repeat pipettor tips (2.5 mL, 5.0 mL, 25.0 mL)	—
Tips, 1000 µL P1000 *Special diameter tips available only from Hologic*	105049
Ten Tube Units (TTU)	TU0022
TTU rack	104579
Ten Tip Cassettes (TTC)	104578
Aptima Specimen Transfer Kit	301154C
Aptima Cervical Specimen Collection and Transport Kit	302657
Bleach, minimum 5% or 0.7 M sodium hypochlorite solution	—
Disposable gloves	—
Aptima Penetrable Caps	105668
Replacement non-penetrable caps	103036A

Optional Materials

	Cat. No.
TECAN Freedom EVO 100/4 Instrument	900932
Aptima Deck Plate Assembly, DTS 800	105200
Tips, 1000 µL conductive, liquid sensing	10612513 (Tecan)
Reagent reservoir (40 mL quarter module)	104765
Split reagent reservoir (19 mL x 2 quarter module)	901172
Bleach Enhancer for Cleaning	302101

DTS Systems Test Procedure

A. Work Area/Equipment Preparation

1. Prior to starting the assay, wipe down work surfaces and pipettors with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Allow solution to contact surfaces and pipettors for at least 1 minute and then follow with a water rinse. Do not allow the solution to dry. Cover the bench surface on which the test will be performed with clean, plastic-backed absorbent laboratory bench covers.
2. Place a sufficient number of Ten Tip Cassettes into the Target Capture System (TCS). Ensure that the TCS wash bottle is filled with Wash Solution and the aspiration manifold is connected to the vacuum pump. Refer to the *Target Capture System Operator's Manual.*
3. Prepare the TECAN Freedom EVO instrument according to the instructions in the Operator's Manual and HPV Application Sheet.
4. Prepare the pre-amplification SB100 instrument according to the instructions in the Operator's Manual and HPV Application Sheet. Turn on the instrument and start the "APTIMA HPV PREAMP" protocol to allow the instrument to warm to 62°C.
5. Upon completion of the amplification step, prepare the post-amplification SB100 instrument according to the instructions in the Operator's Manual and HPV Application Sheet. Turn on the instrument and start the "APTIMA HPV PSTAMP" protocol to allow the instrument to warm to 62°C.
6. Upon completion of the amplification step, prepare the Leader HC+ Luminometer according the instructions in the Operator's Manual after addition of the Probe Reagent as described in the Post Amplification steps.

B. Reagent Reconstitution/Preparation of a New Kit

Note: *Reagent Reconstitution should be performed prior to beginning specimen transfer.*

1. To reconstitute the Aptima HPV 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. Open the lyophilized reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 1, Step 1).
   c. Open the matching reconstitution solution bottle and set the cap on a clean, covered work surface.
   d. 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).

e. Slowly invert the assembled bottle and vial. Allow the solution to drain into the glass container (Figure 1, Step 3).

f. Gently swirl the solution in the vial to mix thoroughly. Avoid creating foam while swirling the vial (Figure 1, Step 4).

g. Wait for the lyophilized reagent to go into solution, then, invert the assembled bottle and vial, tilting at a 45° angle to minimize foam (Figure 1, Step 5). Allow all of the liquid to drain back into the bottle.

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

i. Recap the plastic bottle. Record operator initials and reconstitution date on reconstituted reagent vials (Figure 1, Step 7).

j. Discard both the reconstitution collar and glass vial (Figure 1, Step 8).

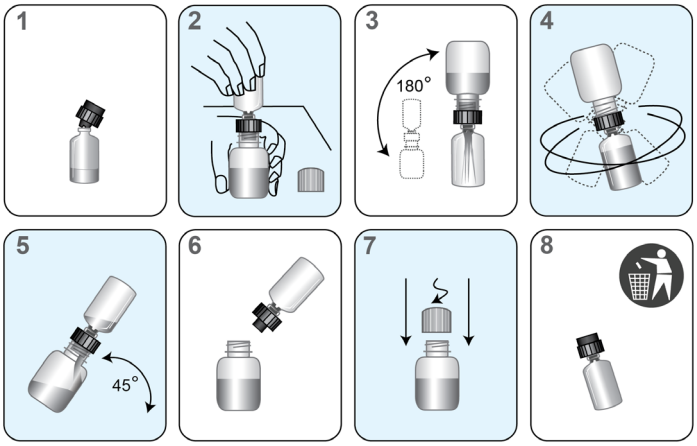

Figure 1. DTS Systems Reconstitution Process

2. Prepare the working Target Capture Reagent (wTCR)
   a. Pair the appropriate bottles of TCR and IC.
   b. Open the bottle of TCR and set the cap on a clean, covered work surface.
   c. Open the bottle of IC and pour the entire contents into a bottle of TCR. A small amount of liquid may remain in the IC vial.
   d. Cap the bottle of TCR and gently swirl the solution to thoroughly mix the contents. Avoid creating foam during this step.
   e. Record the operator's initials and the current date on the label.
   f. Discard the IC bottle and cap.
   g. Precipitate may form in wTCR. Precipitate may be dissolved by warming wTCR at temperatures between 42°C and 60°C for up to 90 minutes. Allow the wTCR to equilibrate to room temperature prior to use.
3. Prepare the Selection Reagent

   If Selection Reagent contains precipitate, warm the Selection Reagent at 60°C ± 1°C for up to 45 minutes to facilitate dissolution of precipitate. Gently mix the bottle every 5 to 10 minutes. Allow the Selection Reagent to equilibrate to room temperature prior to use. Do not use if precipitate or cloudiness persists.

C. Reagent Preparation for Previously Reconstituted Reagents

1. Previously reconstituted Amplification, Enzyme, 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. After re-suspension, mix the vial by gentle inversion. 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.
4. If Selection Reagent contains precipitate, warm the Selection Reagent at 60°C ± 1°C for up to 45 minutes to facilitate dissolution of precipitate. Gently mix the bottle every 5 to 10 minutes. Allow the Selection Reagent to equilibrate to room temperature prior to use. Do not use if precipitate or cloudiness persists.
5. Thoroughly mix each reagent by gently inverting prior to use. Avoid creating foam during inversion of reagents.

D. Rack Setup

1. Allow the samples (calibrators, controls, and specimens) to reach room temperature prior to processing.
2. Do not vortex samples.
3. Inspect sample tubes before piercing them. 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 specimen tube cap.*

4. Place enough TTUs in the TTU rack to accommodate the calibrators, controls, and specimens.
5. (Optional) Create a worklist using the Aptima Worklist Editor Software. Refer to the Worklist Editor section of the *Aptima Assay Software Operator's Manual* for specific instructions.

Manual Pipetting Option

1. Thoroughly mix the wTCR (TCR plus IC). Using a repeat pipettor, add 100 µL of wTCR to each reaction tube.
2. Using a micropipettor, pierce the cap of the sample tube, taking care not to drive the tip into the bottom of the tube.
3. Use a new pipette tip for each calibrator, control, and specimen.
4. Add 400 µL of the Negative Calibrator to the first three tubes in the first TTU.
5. Add 400 µL of the Positive Calibrator to tubes 4-6 of the first TTU.
6. Add 400 µL of the Negative Control to tube 7 of the first TTU.
7. Add 400 µL of the Positive Control to tube 8 of the first TTU.
8. Add 400 µL of each specimen to the remaining tubes.
9. When all samples have been pipetted, cover TTUs with sealing cards and proceed to Target Capture.

TECAN Freedom EVO Instrument Option

Refer to the *TECAN Freedom EVO 100/4 Application Sheet for the Aptima HPV Assay* for specific instructions for the addition of wTCR and samples if using this instrument.

E. Target Capture

For detailed information about the use of the SB100 instrument with the Aptima HPV assay, refer to the *SB100 Dry Heat Bath/Vortexer Application Sheet for the Aptima HPV Assay.*

For information about the use of the Hologic Target Capture System, refer to the *Target Capture System Operator's Manual*.

Note: *The repeat pipettor used in target capture and amplification should be dedicated for use in these steps only. See Tigris DTS System Test Procedure for more information.*

1. Cover the sealing cards with the SB100 frame.
2. Once the SB100 instrument has reached 62°C, holding the frame and rack together to ensure TTUs are locked into position on the rack, ease the rack into the heating block. Take care not to splash contents onto the sealing cards. Rotate the black knobs until the bearings lock into the holes on the frame.
3. Press the appropriate key to start the program.
4. When indicated by the SB100 display upon completion of the last incubation, gently remove the rack from the heating block, taking care not to splash contents onto the sealing cards.
5. Place the rack on the Target Capture System (TCS) magnetic base for 5 to 10 minutes. Perform the following Wash steps:
   a. Prime the Dispense Station pump lines by pumping Wash Solution through the dispense manifold. Pump enough liquid through the system so that there are no air bubbles in the line and all 10 nozzles are delivering a steady stream of liquid.
   b. Turn on the vacuum pump and disconnect the aspiration manifold at the first connector between the manifold and the trap bottle. Ensure that the vacuum gauge meets the leak test specification. It may take 15 seconds to achieve this reading. Reconnect the manifold, and ensure the vacuum gauge meets the vacuum level specification. Leave the vacuum pump on until all target capture steps are completed and the aspiration manifold is dry.
   c. Firmly attach the aspiration manifold to the first set of tips. Aspirate all liquid by lowering the tips into the first TTU until the tips come into brief contact with the bottoms of the tubes. Do not hold the tips in contact with the bottoms of the tubes.
   d. After the aspiration is complete, eject the tips into their original tip cassette. Repeat the aspiration steps for the remaining TTUs, using a dedicated tip for each specimen.
   e. Place the dispense manifold over each TTU and, using the Dispense Station pump, deliver 1.0 mL of Wash Solution into each tube of the TTU.
   f. Cover the tubes with a sealing card and remove the rack from the TCS.
6. Cover the sealing cards with the SB100 frame and ease onto the SB100 heat block. Select the appropriate key to vortex the tubes. After vortexing is complete, remove rack.
7. On the SB100 instrument, press the appropriate key to continue with pre-heating the block.
8. Place the rack back onto the TCS and repeat the aspiration steps in 5c and 5d above.
9. After the final aspiration, remove the rack from the TCS magnetic base and visually inspect the tubes to ensure that all the liquid has been aspirated, and all tubes contain magnetic particle pellets. If any liquid is visible, place the rack back onto the TCS base for 2 minutes, and repeat the aspiration for that TTU using the same tips used previously for each specimen.
10. Proceed to the Amplification step.

F. Amplification

1. Add Amplification Reagent and Oil Reagent.

Manual Pipetting Option

a. Using the repeat pipettor, add 75 µL of the reconstituted Amplification Reagent to each reaction tube. All reaction mixtures in the rack should be red.

b. Using the repeat pipettor, add 200 µL of Oil Reagent.

c. Cover the tubes with sealing cards.

d. Proceed to Step 2.

TECAN Freedom EVO Instrument Option

Refer to the *TECAN Freedom EVO 100/4 Application Sheet for the Aptima HPV Assay* for specific instructions for the addition of Amplification and Oil Reagents if using this instrument.

2. Cover sealing cards with the SB100 frame and ease the rack into the heating block.
3. Press the appropriate key to begin the incubation.
4. When indicated, remove the SB100 frame. Remove and discard sealing cards and add 25 µL of reconstituted Enzyme Reagent using a repeat pipettor, while the rack is still in the heating block.
5. Cover the tubes with new sealing cards and the SB100 frame.
6. Press the appropriate key to begin the amplification incubation.
7. When the incubation step is complete, remove the rack from the SB100 instrument and proceed to the Post-Amplification Step.

G. Post-Amplification

Turn on the post-amplification SB100 instrument and select the “APTIMA HPV PSTAMP” protocol to allow the instrument to warm to 62°C.

For specific information about the use of the SB100 instrument with the Aptima HPV assay, refer to the *SB100 Dry Heat Bath/Vortexer Application Sheet for the Aptima HPV Assay*.

Note: *The repeat pipettor used in detection should be dedicated for use in these steps only. See Warnings and Precautions.*

Note: *Post-amplification steps should be completed in an area separate from the reagent preparation and pre-amplifications steps. See Procedural Notes.*

1. Remove and discard the sealing cards.
2. Using the repeat pipettor add 100 µL of reconstituted Probe Reagent to each reaction tube. All reaction mixtures should be yellow.
3. Cover the tubes with the sealing cards and the SB100 frame and ease the rack into the heating block.
4. Press the appropriate key to start the vortex/incubation steps.
5. When the incubation step is complete, remove the rack and incubate at room temperature for 5 minutes. Be sure to select the appropriate key on the SB100 key pad to start the incubation time.
6. When the 5 minutes is up, as indicated by the SB100 display, add 250 µL Selection Reagent to each reaction tube using the repeat pipettor. All reaction mixtures should be pink.

7. Cover the tubes with sealing cards and the SB100 frame and ease the rack into the heating block. Press the appropriate key to start the vortex/incubation steps.
8. When the incubation is complete, remove the rack from the SB100 instrument and proceed to Detection.

H. Detection

1. The detection step must be performed at 18°C to 28°C.
2. Ensure there are sufficient volumes of Auto Detect 1 and 2 to complete the tests.
3. Prepare the Leader HC+ Luminometer by placing one empty TTU in cassette position number 1 and performing the WASH protocol. Refer to the *Leader HC+ Luminometer Operator's Manual* for specific instructions.
4. Load the TTUs into the luminometer.
5. Log on to the Aptima assay software for HPV. If a worklist was created, ensure that the appropriate pathway is enabled so the Aptima HPV assay software can locate the correct worklist.
6. Click **NEW RUN**. If a worklist was not created enter the number of tubes (Calibrators, Controls and specimens). Click **NEXT** to begin the run.

Note: *The run must be completed within 2 hours of the end of the selection step incubation.*

7. Prepare Deactivation Fluid by mixing equal volumes of 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution and Aptima Buffer for Deactivation Fluid in a large-capped plastic container. Label and write the expiration date on the plastic container. Deactivation Fluid is stable for 4 weeks at room temperature.
8. After removing the used TTUs from the luminometer, place the TTUs into the container with Deactivation Fluid. Allow the TTUs to sit in the container for 15 minutes before disposal. Proper handling and disposal methods should be established by the laboratory director.

Procedural Notes

A. Calibrators

Each run of up to 100 tests must contain three replicates each of the Negative Calibrator and Positive Calibrator. To work properly with the Aptima HPV assay software, the three replicates of the Negative Calibrator followed by the three replicates of the Positive Calibrator must be in the first six positions of the first TTU. Placement in the wrong position will cause the run to fail.

B. Controls

Each run of up to 100 tests must contain one replicate each of the Negative Control and Positive Control. The Negative Control must be in the seventh tube position, followed by the Positive Control in the eighth tube position. Placement in the wrong positions will cause the run to fail.

C. Sample Pipetting

1. The volume of sample added to the reaction tube should be 400 µL ± 100 µL. Visual inspection of the volume pipetted into the TTU is recommended to ensure proper volume transfer. Proper specimen volume is needed to provide accurate results. If the proper volume has not been pipetted, re-pipette the working Target Capture Reagent and the specimen into a new reaction tube.

2. Carefully deliver the samples to each tube avoiding contact with the rim to minimize the chance of carryover from one tube to another.

D. Temperature

1. Room temperature is defined as 15°C to 30°C.
2. Detection is sensitive to temperature. The laboratory temperature in the detection area must be at 18°C to 28°C.

E. Time

The target capture, amplification, hybridization, and selection reactions are all time dependent. Adhere to the times specified in *DTS Systems Test Procedure*.

F. Glove Powder

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

G. Decontamination

1. Laboratory bench surfaces and pipettors must be decontaminated regularly with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Allow the solution to contact surfaces for at least 1 minute, and then follow with a water rinse. Do not allow the solution to dry. Chlorine solutions may pit equipment and metal. Thoroughly rinse equipment with water to avoid pitting.
2. Decontaminate the TECAN Freedom EVO instrument according to the instructions in the Operators Manual.
3. Decontaminate the SB100 instruments per the instructions in the *SB100 Dry Heat Bath/ Vortexer Application Sheet for the Aptima HPV Assay.*
4. Decontaminate the Target Capture System per the instructions in the *Target Capture System Operator's Manual.*
5. Wipe the surfaces of the TCS unit and wash buffer ejector tips with paper towels moistened with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Follow with a water rinse, and then dry the surfaces completely with paper towels.
6. Submerge the TTU racks in 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution, ensuring that they are covered by the solution. Keep the racks submerged for 10 minutes. Longer exposure could damage the racks. Rinse the racks thoroughly with water, place on a clean, absorbent pad, and allow to air-dry thoroughly. To prolong the life of the racks, allow to dry upright, not upside down.
7. TTUs must be decontaminated with Deactivation Fluid as described in the step for Detection. Do not reuse the TTUs.

Tigris DTS System

Reagents for the Aptima HPV assay are listed below for the Tigris DTS System. Reagent Identification Symbols are also listed next to the reagent name.

Reagents and Materials Provided

Aptima HPV Assay Kit, 250 tests, Cat. No. 302611 (4 boxes)

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

Aptima HPV 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 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 Calibrators Box (Cat. No. 302554)
(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 Controls Box (Cat. No. 302556)
(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
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)
Tigris DTS System Run Kit	301191
Multi-tube Units (MTU)	*104772-02*
MTU-Tiplet Waste Bag	*900907*
MTU Waste Deflectors	*900931*
MTU Waste Covers	*105523*
Aptima Specimen Transfer Kit	301154C
Aptima Cervical Specimen Collection and Transport Kit	302657
Aptima Penetrable Caps	105668
Replacement non-penetrable caps	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	—
Aptima Transfer Solution Kit (for SurePath specimens only)	303658

Optional Materials

	Cat. No.
Bleach Enhancer for Cleaning	302101

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 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 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 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 all reconstituted reagent vials (Figure 2, Step 7).

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

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

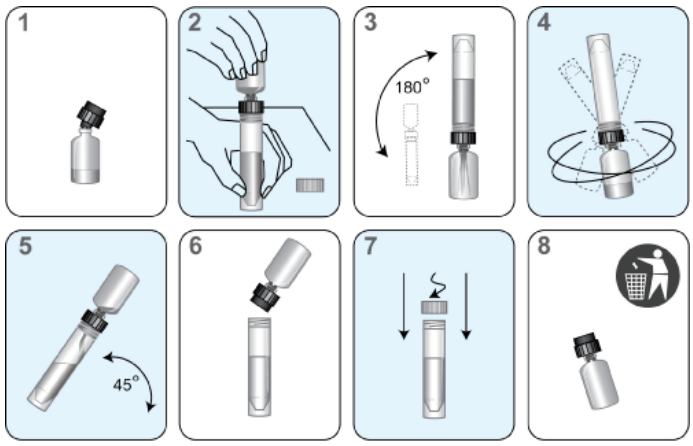

Figure 2. Tigris DTS System Reconstitution Process

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 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 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, and specimens) to reach room temperature prior to processing.
2. **Do not vortex samples**.
3. SurePath liquid cytology specimens must be treated with proteinase K prior to testing with the Aptima HPV assay according to the instructions in the *Specimen Collection and Storage* Section C.
4. 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 4 may result in liquid discharge from the specimen tube cap.*

E. System Preparation

Set up the instrument 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 of 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 for the Aptima HPV assay are listed below for the Panther System. Reagent Identification Symbols are also listed next to the reagent name.

Reagents and Materials Provided

Aptima HPV assay, 250 tests, Cat. No. 303093 (3 boxes)

Aptima HPV assay, 100 tests, Cat. No. 302929 (3 boxes)

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

Aptima HPV 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 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 Calibrators Box (Cat. No. 302554)
(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 Cervical Specimen Collection and Transport Kit	302657
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	—
Aptima Transfer Solution Kit (for SurePath specimens only)	303658

Optional Materials

	Cat. No.
Bleach Enhancer for Cleaning	302101

Panther System Test Procedure

Note: *See the Panther 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 3, Step 1).
   d. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.
   e. While holding the solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle (Figure 3, Step 2).
   f. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 3, Step 3).
   g. Gently swirl the solution in the bottle to mix thoroughly. Avoid creating foam while swirling the bottle (Figure 3, 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 3, Step 5). Allow all of the liquid to drain back into the plastic bottle.
   i. Remove the reconstitution collar and glass vial (Figure 3, Step 6).
   j. Recap the plastic bottle. Record operator initials and the reconstitution date on all reconstituted reagent vials (Figure 3, Step 7).
   k. Discard the reconstitution collar and vial (Figure 3, Step 8).

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

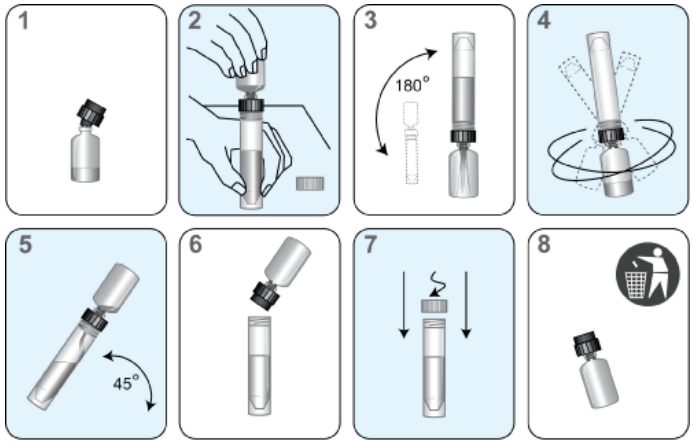

Figure 3. Panther System Reconstitution Process

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 Selection Reagent contains precipitate, warm the Selection Reagent at 60°C ± 1°C for up to 45 minutes to facilitate dissolution of precipitate. Gently mix the bottle every 5 to 10 minutes. Allow the Selection Reagent to equilibrate to room temperature prior to use. Do not use if precipitate or cloudiness persists.

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

C. Reagent Preparation for Previously Reconstituted Reagents

1. Previously reconstituted Amplification, Enzyme, and Probe Reagents, must reach room temperature (15°C to 30°C) prior to the start of the assay.
2. If reconstituted Probe Reagent contains precipitate that does not return to solution at room temperature, heat at a temperature that does not exceed 60°C for 1 to 2 minutes. Do not use if precipitate or cloudiness is present.
3. If wTCR contains precipitate, warm wTCR at 42°C to 60°C for up to 90 minutes. Allow the wTCR to equilibrate to room temperature prior to use. Do not use if precipitate persists.
4. If Selection Reagent contains precipitate, warm the Selection Reagent at 60°C ± 1°C for up to 45 minutes to facilitate dissolution of precipitate. Gently mix the bottle every 5 to 10 minutes. Allow the Selection Reagent to equilibrate to room temperature prior to use. Do not use if precipitate or cloudiness persists.
5. Thoroughly mix each reagent by gently inverting prior to loading 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 and specimens) to reach room temperature prior to processing.
2. **Do not vortex specimens**.
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 specimen tube cap.*

E. System Preparation

1. 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.
2. Load samples.

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 processing errors.

B. Temperature

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

C. Glove Powder

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

Quality Control Procedures

A. Run Validity Criteria

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

- More than one invalid Negative Calibrator replicate.
- More than one invalid Positive Calibrator replicate.
- An invalid Negative Control (DTS Systems and Tigris DTS System only).
- An invalid Positive Control (DTS Systems and 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.

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 Calculation

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

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

D. Analyte Cutoff Calculation

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

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

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

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

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

F. Control Acceptance Criteria (DTS Systems and 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

Assay 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 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. Initial invalid test results should be repeated.

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

Note: *A minimum volume of 1.7 mL is required in order to test 1 aliquot of the sample. Do not dilute an invalid diluted specimen. If a diluted specimen yields an invalid result, a new specimen should be obtained from the patient.*

Aptima HPV 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	*IC > 2,000,000 RLU* *or* *Analyte S/CO < 0.50 and IC < IC Cutoff* *or* *Analyte > 13,000,000 RLU*

Limitations

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

B. The performance of the Aptima HPV assay has not been evaluated for HPV vaccinated individuals.

C. The Aptima HPV assay has not been evaluated in cases of suspected sexual abuse.

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

E. ThinPrep liquid cytology specimens containing less than 1 mL after ThinPrep Pap Test slide preparation are considered inadequate for the Aptima HPV assay.

F. Removal of 1mL of a SurePath liquid cytology specimen prior to cytological processing has not been evaluated for impact to the cytology result.

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

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

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

J. 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.

K. 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.

L. The Aptima HPV assay provides qualitative results. Therefore, a correlation cannot be drawn between the magnitude of a positive assay signal and the expression level of mRNA in a specimen.

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 product 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 carryover rate of the Aptima HPV assay on the Tigris DTS System has been determined in a non-clinical study to be 0.3%.

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

S. 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.

T. The positive control material is not intended to monitor performance at the assay cutoff.

DTS Systems Assay Performance

Aptima HPV Assay Clinical Performance with ThinPrep Liquid Cytology Specimens

Over 700 ThinPrep liquid cytology specimens were collected from European women who were referred for follow-up due to: one or more abnormal Pap tests, an HPV infection, or other reason. One milliliter (1.0 mL) of each specimen was diluted into 2.9 mL of Aptima specimen transport media and a single replicate tested with the Aptima HPV assay. Cytology, histology, and results from a commercially available HPV DNA test (HPV-DNA) were available for most of the specimens. The high-risk HPV status of each specimen was determined by the concordance between the Aptima and the commercially available HPV DNA test and by additional analysis of the specimens with discordant results using an amplified DNA genotyping test. The sensitivity and specificity for detection of HPV nucleic acid was determined. The clinical sensitivity and specificity for the detection of disease, defined as a Cervical Intraepithelial Neoplasia (CIN) 2 or greater histology result, were also calculated for the whole population of specimens as well as specific subsets based on cytological results.

The Aptima HPV assay sensitivity and specificity for detection of high-risk HPV is shown in Table 1 for the 781 specimens tested on the DTS Systems. The sensitivity of the assay was 92.6%, the specificity was 98.5%, and the positive and negative predictive values for detection of high-risk HPV were 98.8% and 90.9%, respectively.

Table 1: Sensitivity and Specificity of the Aptima HPV Assay on the DTS Systems for Detection of High-Risk HPV

		High-Risk HPV		
		+	-	Total
Aptima HPV	+	412	5	417
	-	33	331	364
	Total	445	336	781

Sensitivity (95% CI) = 92.6% (89.8-94.7)
Specificity (95% CI) = 98.5% (96.6-99.4)
Positive Predictive Value = 98.8%
Negative Predictive Value = 90.9%

The Aptima HPV assay clinical sensitivity and specificity for detection of ≥CIN2 is shown in Table 2a for the 753 specimens with histology results tested on the DTS Systems. The clinical sensitivity of the assay was 90.8%, the specificity was 55.7%, and the positive and negative predictive values for detection of ≥CIN2 were 32.1% and 96.3% respectively. Aptima HPV assay sensitivity was similar to HPV-DNA, which was 95.0% (Table 2b), but Aptima HPV assay specificity was significantly higher than HPV-DNA specificity, which was 47.4% in this population for detection of ≥CIN2 lesions. Of the 753 specimens with histology results, 159 had an ASCUS cytology result. The sensitivity and specificity of the Aptima HPV assay in this population was 92.3% and 41.4% respectively for detection of ≥CIN2.

Similar analyses were also performed using a clinical endpoint of ≥CIN3. The Aptima HPV assay clinical sensitivity and specificity for detection of ≥CIN3 is shown in Table 3a for the 753 specimens with histology results tested on the DTS Systems. The clinical sensitivity of the assay was 97.7%, the specificity was 52.9%, and the positive and negative predictive values for detection of ≥CIN3 were 21.3% and 99.4%, respectively. Again, Aptima HPV assay sensitivity was similar to that of HPV-DNA, for which the sensitivity for detection of ≥CIN3 was 98.9% (Table 3b) and Aptima HPV assay specificity was significantly higher than

HPV-DNA specificity, which was 44.4% in this population for detection of ≥CIN3 lesions. Of the 753 specimens with histology results, 159 had an ASCUS cytology result. The sensitivity and specificity of the Aptima HPV assay in this population was 100% and 40.1% respectively for detection of ≥CIN3.

These results, which yielded similar sensitivity and significantly higher specificity for the Aptima HPV assay, as compared to high-risk DNA detection, are similar to results obtained in other studies.[27,28,29,30,31]

Table 2a: Sensitivity and Specificity of the Aptima HPV Assay on the DTS Systems for Detection of Disease (≥CIN2)

		≥CIN2	< CIN2	Total
Aptima HPV	+	128	271	399
	-	13	341	354
	Total	141	612	753

Sensitivity (95% CI) = 90.8% (84.9-94.5)
Specificity (95% CI) = 55.7% (51.8-59.6)
Positive Predictive Value = 32.1%
Negative Predictive Value = 96.3%

Table 2b: Sensitivity and Specificity of the HPV-DNA Test for Detection of Disease (≥CIN2)

		≥CIN2	< CIN2	Total
HPV-DNA	+	134	322	456
	-	7	290	297
	Total	141	612	753

Sensitivity (95% CI) = 95.0% (90.1-97.6)
Specificity (95% CI) = 47.4% (43.5-51.4)
Positive Predictive Value = 29.4%
Negative Predictive Value = 97.6%

Table 3a: Sensitivity and Specificity of the Aptima HPV Assay on the DTS Systems for Detection of Disease (≥CIN3)

		≥CIN3	< CIN3	Total
Aptima HPV	+	85	314	399
	-	2	352	354
	Total	87	666	753

Sensitivity (95% CI) = 97.7% (92.0-99.4)
Specificity (95% CI) = 52.9% (49.1-56.6)
Positive Predictive Value = 21.3%
Negative Predictive Value = 99.4%

Table 3b: Sensitivity and Specificity of the HPV-DNA Test for Detection of Disease (≥CIN3)

		≥CIN3	< CIN3	Total
HPV-DNA	+	86	370	456
	-	1	296	297
	Total	87	666	753

Sensitivity (95% CI) = 98.9% (93.8-99.8)
Specificity (95% CI) = 44.4% (40.7-48.2)
Positive Predictive Value = 18.9%
Negative Predictive Value = 99.7%

Aptima HPV Assay Clinical Performance with Cervical Specimen Collection and Transport Specimens

Paired ThinPrep liquid cytology specimens and Aptima CSCT Kit specimens were collected from 728 subjects. One milliliter (1.0 mL) of each ThinPrep liquid cytology specimen was diluted into 2.9 mL of Aptima specimen transport media and a single replicate tested with the Aptima HPV assay on the DTS Systems. A single replicate of each CSCT specimen was also tested with the Aptima HPV assay. Aptima HPV assay percent agreement between the ThinPrep liquid cytology specimens and the CSCT specimens was determined, and is shown in Table 4.

The percent positive agreement was 95.1% (95% CI: 91.6-97.2); the percent negative agreement was 95.9% (95% CI: 93.7-97.3); and the overall agreement was 95.6% (95% CI: 93.9-96.9). A strong correlation between the liquid Pap and transport kit specimens was observed (kappa = 0.90).

Table 4: Overall Agreement of Aptima HPV Assay Results from ThinPrep Liquid Cytology Specimens and Aptima CSCT Specimens Tested on the DTS Systems

		ThinPrep liquid cytology specimen		
		+	-	Total
Aptima CSCT Kit specimen	+	233	20	253
	-	12	463	475
	Total	245	483	728

Positive agreement = 95.1% (91.6-97.2)
Negative agreement = 95.9% (93.7-97.3)
Overall agreement = 95.6% (93.9-96.9)
Kappa coefficient = 0.90

Analytical Sensitivity

The analytical sensitivity of the Aptima HPV assay for detection of high-risk HPV was determined by testing individual negative clinical ThinPrep liquid cytology specimens spiked with HPV *in vitro* transcripts or infected cells at various concentrations. Thirty replicates of each copy level were tested with each of two reagent lots for a total of 60 replicates. Probit regression analysis was performed and the predicted 95% detection limit determined for each HPV type (Table 5).

Probit regression analysis shows that HPV 16, 18, 31, 33, 35, 39, 45, 56, 58, 59, 66 and 68 had predicted 95% detection limits less than 100 copies/reaction; and types 51 and 52 had predicted 95% detection limits between 100 and 300 copies/reaction.

Table 5: Predicted 95% Detection Limit of the Aptima HPV Assay Determined by Probit Analysis of the DTS Systems Data

Target	95% Detection Limit* (95% Fiducial Limits)
HPV 16	74 (54 - 113)
HPV 18	52 (39 - 76)
HPV 31	19 (14 - 27)
HPV 33	24 (18 - 37)
HPV 35	27 (22 - 38)
HPV 39	32 (23 - 49)
HPV 45	28 (17 - 90)
HPV 51	198 (147 - 289)
HPV 52	239 (187 - 324)
HPV 56	48 (36 - 71)
HPV 58	99 (74 - 146)
HPV 59	89 (68 - 127)
HPV 68	27 (20 - 40)
HPV 66	68 (50 - 105)

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

Assay Reproducibility

The reproducibility of the Aptima HPV assay was determined by testing 16 panel members in triplicate in 2 runs with 2 reagent lots, on 3 instruments by 3 operators. Testing was conducted over 20 days at one site. The panel members are described in Table 6. Six of the panel members were HPV negative (3 were Aptima Specimen Transport Media and 3 were pooled ThinPrep liquid cytology specimens), four were HPV low positive (~95% detection limit), and six were HPV moderate positive (≥ ~ 3x the 95% detection limit). The low positive and moderate positive panel members were comprised of either *in vitro* transcript (IVT) or HPV infected cultured cells in Aptima Specimen Transport Media.

Table 6: Aptima HPV Assay Reproducibility Panel

Panel Member	Description	Concentration	Expected HPV Result
1	*STM Lot 1*	N/A	Negative
2	*SiHa Low Positive*	1 cell/rxn	Positive
3	*HeLa Low Positive*	0.15 cell/rxn	Positive
4	*Clinical Pool 1*	N/A	Negative
5	*ME180 Moderate Positive*	1 cell/rxn	Positive
6	*MS751 Moderate Positive*	1 cell/rxn	Positive
7	*SiHa & HeLa Moderate Positive*	10 cell/rxn & 1 cell/rxn	Positive
8	*STM Lot 2*	N/A	Negative
9	*Clinical Pool 2*	N/A	Negative
10	*HPV 16 IVT Low Positive*	30 copies/rxn	Positive
11	*HPV 18 IVT Low Positive*	30 copies/rxn	Positive
12	*STM Lot 3*	N/A	Negative
13	*HPV 16 IVT Moderate Positive*	100 copies/rxn	Positive
14	*HPV 18 IVT Moderate Positive*	100 copies/rxn	Positive
15	*HPV 16 & HPV 18 Moderate Positive*	100/100 copies/rxn	Positive
16	*Clinical Pool 3*	N/A	Negative

One hundred and eight data points for each reproducibility panel member was analyzed for the DTS Systems, the results for which are summarized in the Table 7. The percent positive for the negative panels ranged from 0 to 3.7; low positive was ≥ 98; and the moderate positive was 100. The agreement with the expected result was > 96% for all of the panel members.

The mean IC S/CO was determined for the 6 negative panel members (1, 4, 8, 9, 12, and 16); the inter-instrument, -operator, -lot, and -run variability was calculated, as well as the intra-run variability. The mean IC S/CO for the negative panel members ranged from 1.76 to 1.92. The coefficient of variation (CV) for the IC S/CO values was quite low, < 10% for all parameters evaluated. The variability of the analyte S/CO values for the negative panel members was not analyzed for the negative panel members due to the inherent variability when values of zero are observed.

The mean analyte S/CO was determined for the 10 positive panel members (2-3, 5-7, 10-11, and 13-15); the inter-instrument, -operator, -lot, and -run variability was calculated, as well as the intra-run variability. The mean analyte S/CO values ranged from 9.00 to 10.70 for the low positive panels and 8.84 to 15.75 for the moderate positive panels. The two panel members containing 2 high-risk HPV types, panel 7 and 15, had mean analyte S/CO values of 22.90 and 23.37 respectively. The CVs for the low positive and moderate positive panel members were < 35% and < 15% respectively, with the highest variability observed within a run. The IC S/CO values were not evaluated for the positive panel members because the IC RLU are not indicative of an individual reaction's performance in an analyte positive sample.

Table 7: Aptima HPV Assay Reproducibility on the DTS Systems

					Mean S/CO		S/CO Variability Analysis*											
							Inter-Instrument		Inter-Operator		Inter-Lot		Inter-Run		Intra-Run		Total	
Panel Member	Description	N	% Positive	Agreement	IC	Analyte	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	*Neg*	108	0.0	100%	1.92	0.00	0.0	0.0	0.0	1.5	0.0	1.9	0.0	0.7	0.1	5.8	0.1	6.3
2	*Low pos*	108	99.1	98.1%	N/A	10.68	0.3	2.6	0.0	0.0	0.4	4.1	0.0	0.0	2.0	19.0	2.1	19.6
3	*Low pos*	108	100	99.1%	N/A	10.65	0.5	4.7	0.0	0.0	0.3	2.5	0.3	3.0	2.4	22.3	2.5	23.1
4	*Neg*	108	0.0	100%	1.80	0.00	0.0	2.1	0.0	1.8	0.0	0.2	0.0	0.7	0.1	6.6	0.1	7.2
5	*Mod pos*	107^	100	100%	N/A	8.84	0.2	1.8	0.1	0.8	0.2	2.3	0.0	0.0	0.6	7.2	0.7	7.8
6	*Mod pos*	108	100	100%	N/A	15.75	0.4	2.4	0.4	2.6	1.1	7.0	0.1	0.9	0.6	3.9	1.4	8.7
7	*Mod pos*	107^	100	100%	N/A	22.90	0.7	3.2	0.0	0.0	0.0	0.0	0.0	0.0	2.1	9.1	2.2	9.7
8	*Neg*	108	0.0	100%	1.85	0.00	0.0	0.0	0.0	2.2	0.0	1.1	0.0	1.5	0.1	6.1	0.1	6.8
9	*Neg*	108	3.7	96.3%	1.76	0.06	0.0	0.0	0.1	3.6	0.0	0.0	0.0	1.3	0.1	7.5	0.1	8.4
10	*Low pos*	108	99.1	99.1%	N/A	10.61	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	16.8	1.8	16.8
11	*Low pos*	108	98.1	98.1%	N/A	9.04	0.0	0.0	0.4	4.1	0.0	0.0	0.9	10.0	2.9	32.6	3.1	34.3
12	*Neg*	108	0.0	100%	1.85	0.00	0.0	0.0	0.0	0.0	0.0	1.3	0.0	1.0	0.1	7.6	0.1	7.8
13	*Mod pos*	108	100	100%	N/A	10.99	0.1	1.4	0.1	0.8	0.0	0.2	0.0	0.0	0.4	3.9	0.5	4.2
14	*Mod pos*	108	100	100%	N/A	12.22	0.3	2.6	0.0	0.0	0.0	0.0	0.0	0.0	1.6	12.8	1.6	13.0
15	*Mod pos*	108	100	100%	N/A	23.37	0.7	2.8	0.3	1.5	0.0	0.0	0.1	0.6	2.5	10.5	2.6	11.0
16	*Neg*	108	0.9	99.1%	1.79	0.03	0.0	2.3	0.0	1.7	0.0	0.0	0.0	1.1	0.1	7.5	0.1	8.1

*IC S/CO variability analysis for the negative panels (1, 4, 8, 9, 12, 16); analyte S/CO variability analysis for the positive panels (2, 3, 5, 6, 7, 10, 11, 13, 14, 15)

^1 invalid reaction not retested

S/CO= signal to cut-off ratio SD= standard deviation N/A= not applicable

Cross-Reactivity

The analytical specificity of the Aptima HPV assay was evaluated with PreservCyt solution media diluted into Aptima specimen transport media and spiked with cultured bacteria, yeast, or fungi; cultured virus; or low-risk HPV *in vitro* transcripts. The analytical sensitivity was evaluated with the same panel spiked with a low concentration of HPV infected SiHa cells (1 cell per reaction). The organisms and test concentrations are identified in Table 8. No effect on Aptima HPV assay specificity or sensitivity was observed with any of the organisms tested.

Table 8: Analytical Specificity Panel

Organism	Test Concentration	Organism	Test Concentration
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*	$5x10^8$ CFU/mL 1.5x104 TCID 50/mL
Campylobacter fetus-fetus	$1x10^8$ CFU/mL	*Neisseria meningitidis*	$1x10^8$ CFU/mL
Chlamydia trachomatis	$2x10^4$ 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
Lactobacillus jensenii	$1x10^8$ CFU/mL		

Table 8: Analytical Specificity Panel *(continued)*

Organism	Test Concentration	Organism	Test Concentration
Yeast/protozoa			
Candida albicans	$1x10^8$ CFU/mL	*Trichomonas vaginalis*	$1x10^7$ cells/mL
Viruses			
Adenovirus 2	$1x10^6$ vp/mL	Herpes simplex virus 1	$2.5x10^5$ TCID 50/mL
Cytomegalovirus	33 TCID 50/mL	Herpes simplex virus 2	$5x10^4$ TCID 50/mL
Epstein-Barr virus	$4x10^7$ 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 53	$2.5x10^6$ copies/mL
HPV 11	$2.5x10^6$ copies/mL	HPV 61	$2.5x10^6$ copies/mL
HPV 42	$2.5x10^6$ copies/mL	HPV 71	$2.5x10^6$ copies/mL
HPV 43	$2.5x10^6$ copies/mL	HPV 81	$2.5x10^6$ copies/mL
HPV 44	$2.5x10^6$ copies/mL		

Interference

The substances described in Table 9 were individually spiked into PreservCyt solution and Aptima Specimen Transport Media (STM) at 1% and 10% v/v or w/v and tested with the Aptima HPV assay. All substances were tested in the presence and absence of HPV infected cultured cells (SiHa, 3 cells/reaction). Interference was not observed with any of the substances tested, except with two of the five lubricants that contained Polyquaternium 15 at concentrations > 0.025% in the test sample, and an anti-fungal medication containing tioconazole at concentrations > 0.075% in the test sample.

Table 9: Substances Tested for Possible Interference with the Aptima HPV Assay

Product Category	Product Brand or Type
Lubricant	KY Sensual Mist (v/v)
	KY Warming Jelly (w/v)
	KY Warming Liquid (v/v)
	Astroglide Personal Lubricant*
	Target Brand Lubricating Liquid*
Spermicide	Gynol II Vaginal Contraceptive Original Formula (w/v)
	Gynol II Vaginal Contraceptive Extra Strength (w/v)
	Delfen Vaginal Contraceptive Foam (w/v)
	Encare Vaginal Contraceptive (w/v)
	Conceptrol Vaginal Contraceptive (w/v)
Anti-fungal/Anti-Itch Medication	Vagisil Maximum Strength (w/v)
	Monistat Soothing Care (w/v)
	Monistat 3 Combination Pack (w/v)
	Target Brand Tioconazole 1 (w/v)
	Target Brand Miconazole 3 (w/v)
Glacial Acetic Acid	EMD M/N AX0073-11 (v/v)
Whole Blood	whole blood (v/v)

*Personal lubricants that contain Polyquaternium 15.

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.[32,33] 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.[34] 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 10 for the ASC-US (atypical squamous cells of undetermined significance) and the NILM (negative for intraepithelial lesion or malignancy) populations.

Table 10: 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 Clinical Study Design with ThinPrep Liquid Cytology Specimens

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). The CLEAR trial included a baseline evaluation and a 3 year follow-up evaluation.[34]

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.[35]

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 a commercially available 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 commercially available HPV DNA test, as well as randomly selected women who were negative with both assays, were referred to colposcopy for the baseline evaluation. 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 commercially available 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 a commercially available 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 with ThinPrep Liquid Cytology Specimens

In total, there were 1,252 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 11.

Table 11: 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 12, as are the estimates for the commercially available HPV DNA test.

Table 12: ASC-US ≥ 21 Years Population: Performance of the Aptima HPV Assay and an 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 commercially available HPV DNA test, where both assay results are available for the detection of ≥CIN2 and ≥CIN3, were similar (differences in sensitivity estimates were not statistically significant: sensitivity difference = -2.3% [95% CI: -9.5%, 4.8%]). Clinical specificity estimates of the Aptima HPV assay for the detection of ≥CIN2 and ≥CIN3 were higher than those of the commercially available 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 commercially available 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.[36,37] 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 commercially available HPV DNA test.

Clinical performance estimates of the Aptima HPV assay and the commercially available HPV DNA test are shown by age group in Table 13 and Table 14 (≥CIN2 and ≥CIN3, respectively, based on evaluating all biopsies).

Table 13: ASC-US ≥ 21 Years Population: Performance of the Aptima HPV Assay and an 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 14: ASC-US ≥ 21 Years Population: Performance of the Aptima HPV Assay and an 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 15, as are the estimates for the commercially available 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 15: ASC-US ≥ 21 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an 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 commercially available HPV DNA test are shown by age group in Table 16.

Table 16: ASC-US ≥ 21 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an 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 with ThinPrep Liquid Cytology Specimens 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 17.

Table 17: NILM ≥ 30 Years Population: Results of the Aptima HPV Assay and an 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 18). Because only randomly selected women with negative results for both the Aptima HPV assay and the commercially available HPV DNA test were referred to colposcopy, the proportion of women with unverified disease status was high in this group (96.6%). To adjust for this verification bias, a multiple imputation method was used to estimate the number of women with disease that would have been identified if all women had undergone colposcopy. Both verification-bias adjusted performance estimates and unadjusted performance estimates based on the 819 women with verified disease status at baseline are presented.

Table 18: NILM ≥ 30 Years Population: Classification of Evaluable NILM Women by Aptima HPV Assay and 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 19. 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 20 and by age group in Table 21.

Table 19: NILM ≥ 30 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an HPV DNA Test (Verification-Bias Adjusted Estimates) at Baseline

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

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	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 21: NILM ≥ 30 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an 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 22, as are the estimates for the commercially available HPV DNA test. Unadjusted clinical performance estimates are shown in Table 23. The Aptima HPV assay and the commercially available 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 commercially available 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 commercially available HPV DNA test (4.7% vs 3.7%).

Table 22: NILM ≥ 30 Years Population: Performance of the Aptima HPV Assay and an 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 23: NILM ≥ 30 Years Population: Performance of the Aptima HPV Assay and an 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 commercially available HPV DNA test demonstrates similar sensitivity and statistically significant improved specificity of the Aptima HPV assay over the commercially available HPV DNA test for detection of ≥CIN2 as shown by the ratios of true positive and false positive rates (Table 24 and Table 25, respectively).

Table 24: NILM ≥ 30 Years Population: Ratio of True Positive Rates (Aptima HPV Assay/HPV DNA Test) for Women with ≥CIN2 (Unadjusted Estimates) at Baseline

		HPV DNA Test		Total
		Positive	Negative	
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 25: NILM ≥ 30 Years Population: Ratio of False Positive Rates (Aptima HPV Assay/ HPV DNA Test) for Women with <CIN2 (Unadjusted Estimates) at Baseline

		HPV DNA Test		Total
		Positive	Negative	
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 a commercially available HPV DNA assay, and the 3-year disease status (includes baseline and follow-up evaluation) by Consensus Histology Review Panel are presented in Table 26.

Table 26: NILM ≥ 30 Years Population: Classification of Women Eligible for the Follow-up Phase by Baseline Aptima HPV Assay Results, Baseline 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 27.

Table 27: NILM ≥ 30 Years Population: 3-Year Cumulative Absolute and Relative Risks* of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an 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 Clinical Performance with SurePath Liquid Cytology Specimens

SurePath Specimens Treated with Aptima Transfer Solution

SurePath liquid cytology specimens were collected from Canadian women (n=558) who were referred for follow-up due to one or more abnormal Pap tests, an HPV infection, or some other reason. An aliquot (0.5 mL) of each specimen was transferred into an Aptima Specimen Transfer tube and then treated using the Aptima Transfer Solution. A single replicate of each specimen was tested with the Aptima HPV assay. A separate aliquot (1 mL) of each specimen was removed for evaluation with a commercially available HPV PCR test. The clinical sensitivity for the detection of disease, defined as a ≥CIN3 histology result, was calculated for both the Aptima HPV assay and the HPV PCR test, as shown in Table 28, with the positive and negative predictive values.

Table 28: Performance of the Aptima HPV Assay and an HPV PCR Test for Detection of ≥CIN3

Performance	Aptima HPV Assay N=558		HPV PCR Test N=558	
	Estimate	(95% CI)	Estimate	(95% CI)
Sensitivity (%)	89.3 (25/28)	(72.8 - 96.3)	89.3 (25/28)	(72.8 - 96.3)
Specificity (%)	56.8 (301/530)	(52.5 - 60.9)	49.1 (260/530)	(44.8 - 53.3)
PPV (%)	9.8 (25/254)	(8.1 - 11.2)	8.5 (25/295)	(7.0 - 9.5)
NPV (%)	99.0 (301/304)	(97.6 - 99.8)	98.9 (260/263)	(97.2 - 99.7)
Prevalence (%)	5.0 (28/558)		5.0 (28/558)	

Table 29: Aptima HPV Assay Sensitivity with SurePath and ThinPrep Liquid Cytology Specimens

HPV Genotype	Copies/ reaction	ThinPrep	SurePath
		% Positive (95% CI)	% Positive (95% CI)
16	60	98.3 (91.1-99.7)	100 (94.0-100)
18	100	100 (94.0-100)	100 (94.0-100)
31	25	100 (94.0-100)	95.0 (86.3-98.3)
33	60	96.7 (88.6-99.1)	98.3 (91.1-99.7)
35	25	100 (94.0-100)	100 (94.0-100)
39	25	100 (94.0-100)	91.7 (81.9-96.4)
45	40	100 (94.0-100)	95.0 (86.3-98.3)
51	250	100 (94.0-100)	100 (94.0-100)
52	600	100 (94.0-100)	98.3 (91.1-99.7)
56	100	98.3 (91.1-99.7)	93.3 (84.1-97.4)
58	50	95.0 (86.3-98.3)	93.3 (84.1-97.4)
59	75	96.7 (88.6-99.1)	91.7 (81.9-96.4)
66	150	98.3 (91.1-99.7)	95.0 (86.3-98.3)
68	30	96.7 (88.6-99.1)	93.3 (84.1-97.4)

Aptima HPV Assay Performance with Cervical Specimen Collection and Transport Specimens

Paired ThinPrep liquid cytology specimens and Aptima CSCT Kit specimens were collected from 735 subjects. One milliliter (1.0 mL) of each ThinPrep liquid cytology specimen was diluted into 2.9 mL of Aptima specimen transport media and a single replicate tested with the Aptima HPV assay on the Tigris DTS System. A single replicate of each CSCT specimen was also tested with the Aptima HPV assay. Aptima HPV assay percent agreement between the ThinPrep liquid cytology specimen and the CSCT specimen was determined and the results are shown in Table 30.

The percent positive agreement was 95.9% (95% CI: 92.6-97.8); the percent negative agreement was 95.5% (95% CI: 93.3-97.0); and the overall agreement was 95.6% (95% CI: 93.9-96.9). A strong correlation between the liquid cytology and transport kit specimens was observed (kappa = 0.90).

Table 30: Overall Agreement of Aptima HPV Assay Results From ThinPrep Liquid Cytology Specimens and Aptima Cervical Specimen Collection and Transport Kit Specimens Tested on the Tigris DTS System

		ThinPrep liquid cytology specimen		
		Positive	Negative	**Total**
Aptima CSCT Kit specimen	Positive	234	22	256
	Negative	10	469	479
	Total	244	491	735

Positive agreement = 95.9% (92.6-97.8)
Negative agreement = 95.5% (93.3-97.0)
Overall agreement = 95.6% (93.9-96.9)
Kappa coefficient = 0.90

Analytical Sensitivity

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 testing 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 31, show that HPV 16, 18, 31, 33, 35, 38, 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 31: 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 to determine assay reproducibility. Study 2 was conducted in-house to measure assay repeatability. 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 32a (panel members with expected positive results) and Table 32b (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 33 for Study 1 and Table 34 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 32a: Aptima HPV Assay Reproducibility 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)	% positive agreement (95% CI)
HPV 16 & HPV 18 IVT (100 copies)	100 (161/161) (97.7, 100)	100 (162/162) (97.7, 100)
SiHa cells (3 cells) & HeLa cells (7.5 cells)	100 (162/162) (97.7, 100)	100 (162/162) (97.7, 100)
HPV 18 IVT (100 copies)	100 (162/162) (97.7, 100)	100 (160/160) (97.7, 100)
HPV 16 IVT (100 copies)	100 (162/162) (97.7, 100)	100 (162/162) (97.7, 100)
MS751 cells (1 cell)	99.4 (161/162) (96.6, 99.9)	96.9 (157/162) (93.0, 98.7)
ME180 cells (0.3 cells)	95.1 (154/162) (90.6, 97.5)	93.2 (151/162) (88.3, 96.2)
HPV 18 IVT (30 copies)	99.4 (161/162) (96.6, 99.9)	100 (162/162) (97.7, 100)
HPV 16 IVT (30 copies)	100 (162/162) (97.7, 100)	97.5 (158/162) (93.8, 99.0)
HeLa cells (2.5 cells)	100 (162/162) (97.7, 100)	95.6 (152/159) (91.2, 97.9)
SiHa cells (1 cell)*	77.2 (125/162) (70.1, 83.0)	79.0 (128/162) (72.1, 84.6)

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 32b: Aptima HPV Assay Reproducibility 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)	% negative agreement (95% CI)
HPV 18 IVT (1 copy)*	78.8 (126/160) (71.8, 84.4)	83.3 (135/162) (76.8, 88.3)
HPV 16 IVT (1 copy)*	80.9 (131/162) (74.1, 86.2)	88.3 (143/162) (82.4, 92.4)
HeLa cells (0.05 cells)*	79.0 (128/162) (72.1, 84.6)	82.1 (133/162) (75.5, 87.2)
SiHa cells (0.03 cells)*	93.8 (152/162) (89.0, 96.6)	95.7 (155/162) (91.4, 97.9)
STM Lot 1	100 (162/162) (97.7, 100)	100 (162/162) (97.7, 100)
STM Lot 2	99.4 (160/161) (96.6, 99.9)	100 (162/162) (97.7, 100)
STM Lot 3	99.4 (161/162) (96.6, 99.9)	99.4 (161/162) (96.6, 99.9)
ThinPrep Pool 1	97.5 (158/162) (93.8, 99.0)	97.5 (158/162) (93.8, 99.0)
ThinPrep Pool 2	96.9 (157/162) (93.0, 98.7)	96.3 (156/162) (92.2, 98.3)
ThinPrep Pool 3	100 (162/162) (97.7, 100)	99.4 (161/162) (96.6, 99.9)

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

Table 33: Aptima HPV Assay Reproducibility 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^	23.4	0.1	0.4	0.1	0.4	0.9	4.0	0	0	1.6	7.0	1.9	8.1
SiHa cells (3 cells) & HeLa cells (7.5 cells)	162	17.9	0	0	1.4	8.1	0	0	0.6	3.1	5.1	28.6	5.3	29.9
HPV 18 IVT (100 copies)	162	11.8	0	0	0	0	0.8	6.4	0.1	0.9	1.2	10.1	1.4	12.0
HPV 16 IVT (100 copies)	162	10.8	0.2	1.5	0	0	0.1	1.1	0.3	2.6	0.3	3.1	0.5	4.5
MS751 cells (1 cell)	162	13.3	0.3	2.1	0	0	1.0	7.8	0.9	7.1	2.2	16.2	2.6	19.4
ME180 cells (0.3 cells)	162	6.5	0.2	3.2	0	0	0.6	8.6	0.4	5.5	2.4	36.2	2.5	37.7
HPV 18 IVT (30 copies)	162	9.0	0.7	7.3	0	0	0.7	7.2	0.8	8.3	2.3	25.3	2.6	28.5
HPV 16 IVT (30 copies)	162	10.8	0.1	0.8	0	0	0.1	1.3	0.4	3.8	0.9	8.4	1.0	9.3
HeLa cells (2.5 cells)	162	12.4	0	0	0.4	3.3	0.4	3.1	0	0	2.3	18.4	2.4	19.0
SiHa cells (1 cell)	162	7.5	0.3	3.7	1.0	13.0	0	0	0	0	4.8	63.6	4.9	65.0

SD = standard deviation; CV = coefficient of variation; IVT = *in vitro* transcript; S/CO = signal to cutoff ratio
^One sample had an invalid Aptima HPV assay result and was 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 0.*

Table 34: Aptima HPV Assay Reproducibility 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	23.2	0.4	1.5	0.6	2.3	0.8	3.4	0.8	3.4	1.5	6.3	2.0	8.4
SiHa cells (3 cells) & HeLa cells (7.5 cells)	162	18.6	0	0	1.7	9.3	0	0	3.5	18.6	3.7	20.0	5.4	28.9
HPV 18 IVT (100 copies)	160^	11.9	0.1	0.6	0.2	1.6	0.8	7.0	0.4	3.6	1.3	11.3	1.7	13.8
HPV 16 IVT (100 copies)	162	10.8	0	0	0.1	1.3	0	0	0.2	2.2	0.7	6.1	0.7	6.6
MS751 cells (1 cell)	162	13.6	0	0	0.6	4.3	0	0	2.5	18.4	2.1	15.2	3.3	24.2
ME180 cells (0.3 cells)	162	5.8	0	0	0.6	10.8	0.5	9.4	2.2	36.9	1.7	29.7	2.9	49.5
HPV 18 IVT (30 copies)	162	8.8	0.4	4.4	0.5	6.0	0.7	7.9	1.0	11.5	1.9	21.4	2.4	26.6
HPV 16 IVT (30 copies)	162	10.5	0	0	0.1	1.3	0.2	2.0	1.6	14.9	1.2	11.2	2.0	18.8
HeLa cells (2.5 cells)	159^	12.0	0.6	5.1	1.0	8.5	0	0	2.8	23.8	2.0	16.6	3.7	30.6
SiHa cells (1 cell)	162	7.4	0.9	12.5	0	0	0.7	9.3	1.8	24	4.2	56.8	4.7	63.8

SD = standard deviation; CV = coefficient of variation; IVT = *in vitro* transcript; S/CO = signal to cutoff ratio
^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 0.*

A third study was also conducted to determine assay reproducibility 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 35, 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 36.

Agreement was 100% for the high HPV-positive panel members, ≥ 98.6% for the low HPV-positive panel members, and ≥ 94.4% were for the HPV-negative panel members.

Table 35: Aptima HPV Assay Reproducibility Study 3: Panel Description, Percent Agreement

Panel Description	% agreement (95% CI)
Low positive 1	98.6 (71/72) (92.5, 99.8)
Low positive 2	100 (72/72) (94.9, 100)
High positive 1	100 (72/72) (94.9, 100)
High positive 2	100 (72/72) (94.9, 100)
Negative 1	98.6 (71/72) (92.5, 99.8)
Negative 2	94.4 (68/72) (86.6, 97.8)

Table 36: Aptima HPV Assay Reproducibility 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	9.8	0	0	0	0	0	0	2.2	22.8	3.0	30.4	3.7	38.0
Low positive 2	72	10.5	0	0	2.2	21.0	0.9	9.0	3.7	35.3	2.7	26.1	5.2	49.5
High positive 1	72	22.7	1.3	5.6	0	0	0.1	0.5	3.0	13.3	3.7	16.4	5.0	21.9
High positive 2	72	23.9	0	0	0	0	0	0	2.9	12.3	3.0	12.4	4.2	17.4

SD = standard deviation; CV = coefficient of variation; S/CO = signal to cutoff ratio

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 0.*

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 37. 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 37: 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
Lactobacillus jensenii	$1x10^8$ CFU/mL		

Table 37: Analytical Specificity Panel: Organisms and Concentration with No Cross-Reactivity *(continued)*

Organism	Test Concentration with No Cross-Reactivity	Organism	Test Concentration with No Cross-Reactivity
Yeast/protozoa			
Candida albicans	$1x10^8$ 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 37, 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 38 were individually spiked into PreservCyt solution at 1% and 10% v/v or w/v, diluted with STM and then tested with 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 38: 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

*Personal lubricants that contain Polyquaternium 15.

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.[32,33] 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.[34] 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 39 for the ASC-US (atypical squamous cells of undetermined significance) and the NILM (negative for intraepithelial lesion or malignancy) populations.

Table 39: 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 Clinical Study Design with ThinPrep Liquid Cytology Specimens

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.[34]

CLEAR Trial – Baseline Evaluation

The CLEAR trial was conducted to determine the clinical performance of the Aptima HPV assay on the Tigris DTS System for detection of cervical intraepithelial neoplasia grade 2 or more severe cervical disease (≥CIN2). The CLEAR Trial included a baseline evaluation and a 3 year follow-up evaluation. Women were enrolled into either the ASC-US Study or the NILM Study based on 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.[35]

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 DTS System and a commercially available 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 commercially available HPV DNA test, as well as randomly selected women who were negative with both assays, were referred to colposcopy for the baseline evaluation. 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 commercially available 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 a commercially available 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 1,252 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 40.

Table 40: 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 41, as are the estimates for the commercially available HPV DNA test.

Table 41: ASC-US ≥ 21 Years Population: Performance of the Aptima HPV Assay and an HPV DNA Test for Detection of ≥CIN2 and ≥CIN3

	Performance	Aptima HPV Assay N=937		HPV DNA Test N=863*	
		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 commercially available 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 commercially available 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 commercially available 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.[36,37] 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 commercially available HPV DNA test.

Clinical performance estimates of the Aptima HPV assay and the commercially available HPV DNA test are shown by age group in Table 42 and Table 43 (≥CIN2 and ≥CIN3, respectively, based on evaluating all biopsies).

Table 42: ASC-US ≥ 21 Years Population: Performance of the Aptima HPV Assay and an 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 43: ASC-US ≥ 21 Years Population: Performance of the Aptima HPV Assay and an 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 44, as are the estimates for the commercially available 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 44: ASC-US ≥ 21 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an 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 commercially available HPV DNA test are shown by age group in Table 45.

Table 45: ASC-US ≥ 21 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an HPV DNA Test by Age Group

	Age	Assay Result	Aptima HPV Assay N=937		HPV DNA Test N=863*	
			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 with ThinPrep Liquid Cytology Specimens 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 46.

Table 46: NILM ≥ 30 Years Population: Results of the Aptima HPV Assay and an 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 47). Because only randomly selected women with negative results for both the Aptima HPV assay on the Tigris DTS System and the commercially available HPV DNA test were referred to colposcopy, the proportion of women with unverified disease status was high in this group (96.6%). To adjust for this verification bias, a multiple imputation method was used to estimate the number of women with disease that would have been identified if all women had undergone colposcopy. Both verification-bias adjusted performance estimates and unadjusted performance estimates based on the 818 women with verified disease status at baseline are presented.

Table 47: NILM ≥ 30 Years Population: Classification of Evaluable NILM Women by Aptima HPV Assay and an 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	9,032 (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 48. 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 49 and by age group in Table 50.

Table 48: NILM ≥ 30 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an 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 49: NILM ≥ 30 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an 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 50: NILM ≥ 30 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an 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 51, as are the estimates for the commercially available HPV DNA test. Unadjusted clinical performance estimates are shown in Table 52. The Aptima HPV assay and the commercially available 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 commercially available 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 commercially available HPV DNA test (4.5% vs 3.7%).

Table 51: NILM ≥ 30 Years Population: Performance of the Aptima HPV Assay and an 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 52: NILM ≥ 30 Years Population: Performance of the Aptima HPV Assay and an 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 commercially available HPV DNA test demonstrates similar sensitivity and statistically significant improved specificity of the Aptima HPV assay over the commercially available HPV DNA test for detection of ≥CIN2 as shown by the ratios of true positive and false positive rates (Table 53 and Table 54, respectively).

Table 53: NILM ≥ 30 Years Population: Ratio of True Positive Rates (Aptima HPV Assay/HPV DNA Test) for Women with ≥CIN2 (Unadjusted Estimates) at Baseline

		HPV DNA Test		Total
		Positive	Negative	
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 54: NILM ≥ 30 Years Population: Ratio of False Positive Rates (Aptima HPV Assay/HPV DNA Test) for Women with <CIN2 (Unadjusted Estimates) at Baseline

		HPV DNA Test		Total
		Positive	Negative	
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 the

commercially available HPV DNA assay, and the 3-year disease status (includes baseline and follow-up evaluation) by Consensus Histology Review Panel are presented in Table 55.

Table 55: NILM ≥ 30 Years Population: Classification of Women Eligible for the Follow-up Phase by Baseline Aptima HPV Assay Results, Baseline 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 56.

Table 56: NILM ≥ 30 Years Population: 3-Year Cumulative Absolute and Relative Risks* of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an 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% CI 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).

Aptima HPV Assay Clinical Performance with SurePath Liquid Cytology Specimens

SurePath liquid cytology specimens were collected from Canadian women (n=558) who were referred for follow-up due to one or more abnormal Pap tests, an HPV infection, or some other reason. An aliquot (0.5 mL) of each specimen was transferred into an Aptima Specimen Transfer tube and then treated using the Aptima Transfer Solution. A single replicate of each specimen was tested with the Aptima HPV assay. A separate aliquot (1 mL) of each specimen was removed for evaluation with a commercially available HPV PCR test. The clinical sensitivity for the detection of disease, defined as a ≥CIN3 histology result, was calculated for both the Aptima HPV assay and the HPV PCR test, as shown in Table 57, with the positive and negative predictive values.

Table 57: Performance of the Aptima HPV Assay and an HPV PCR Test for Detection of ≥CIN3

Performance	Aptima HPV Assay N=558		HPV PCR Test N=558	
	Estimate	(95% CI)	Estimate	(95% CI)
Sensitivity (%)	89.3 (25/28)	(72.8 - 96.3)	89.3 (25/28)	(72.8 - 96.3)
Specificity (%)	58.7 (311/530)	(54.4 - 62.8)	49.1 (260/530)	(44.8 - 53.3)
PPV (%)	10.2 (25/244)	(8.4 - 11.7)	8.5 (25/295)	(7.0 - 9.5)
NPV (%)	99.0 (311/314)	(97.6 - 99.8)	98.9 (260/263)	(97.2 - 99.7)
Prevalence (%)	5.0 (28/558)		5.0 (28/558)	

Aptima HPV Assay Performance with Cervical Specimen Collection and Transport Specimens

High-risk HPV-positive and high-risk HPV-negative clinical specimens collected from both screening (routine visit) and referral (colposcopy visit) populations with the Aptima CSCT Kit were tested with the Aptima HPV Assay on the Panther and Tigris DTS Systems using two reagent lots. Agreement between the Panther and Tigris DTS Systems for CSCT specimens are shown in Table 58.

For CSCT specimens, overall agreement between the Panther and Tigris DTS Systems was > 98%, as shown in Table 58. Of the 632 clinical specimens tested, 69 were CIN2+ and 38 were CIN3+. The Aptima HPV assay sensitivity for detection of CIN2+ was 97.1% (95% C.I. 90.0%-99.2%) on the Panther System and 98.6% (95% CI: 92.2-99.7) on the Tigris DTS System. Sensitivity for detection of CIN3+ was 100% (CI: 90.8%-100%) on both Panther and Tigris DTS Systems.

Table 58: Agreement of Aptima HPV Assay Results From Aptima CSCT Specimens Tested on the Tigris DTS and Panther Systems

		Tigris DTS System		
		Positive	**Negative**	**Total**
Panther System	**Positive**	490	3	493
	Negative	9	130	139
	Total	499	133	632

Overall Agreement = 98.1% (CI 96.7-98.9)
Positive Agreement = 98.2% (CI 96.6-99.0)
Negative Agreement = 97.7% (CI 93.6-99.2)

Analytical Sensitivity

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 59 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 59: 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 60a (panel members with expected positive results) and Table 60b (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 61 for Study 1 and Table 62 for Study 2.

Table 60a: 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)	% positive agreement (95% CI)
HPV high positive clinical sample 1	100 (107/107) (96.5, 100)	100 (161/161) (97.7, 100)
HPV high positive clinical sample 2	100 (107/107) (96.5, 100)	100 (162/162) (97.7, 100)
HPV 16 IVT (1830 copies)	100 (107/107) (96.5, 100)	100 (161/161) (97.1, 100)
HPV 18 IVT (1550 copies)	100 (107/107) (96.5, 100)	100 (162/162) (97.7, 100)
HPV low positive clinical sample 1	94.4 (101/107) (88.3, 97.4)	89.5 (145/162) (83.3, 93.3)
HPV low positive clinical sample 2	88.0 (95/108) (80.5, 92.8)	92.0 (149/162) (86.8, 95.3)
HPV low positive clinical sample 3	100 (108/108) (96.6, 100)	97.5 (157/161) (93.8, 99.0)
HPV low positive clinical sample 4	90.7 (98/108) (83.8, 94.9)	92.6 (150/162) (87.5, 95.7)
HPV 16 IVT (183 copies)	100 (102/102) (96.4, 100)	100 (162/162) (97.7, 100)
HPV 18 IVT (155 copies)	100 (108/108) (96.6, 100)	100 (159/159) (97.6, 100)
MS751 cells (0.63 cells)	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
HeLa cells (0.35 cells)	100 (108/108) (96.6, 100)	100 (162/162) (97.7, 100)
SiHa cells (0.90 cells)	87.9 (94/107) (80.3, 92.8)	89.5 (145/162) (83.8, 93.3)

IVT = in vitro transcript

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

Table 60b: 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)	% negative agreement (95% CI)
MS751 cells (0.005 cells)	87.0 (94/108) (79.4, 92.1)	93.8 (152/162) (89.0, 96.6)
SiHa cells (0.008 cells)	97.2 (105/108) (92.1, 99.1)	95.7 (155/162) (91.4, 97.9)
HeLa cells (0.02 cells)	70.4 (76/108) (61.2, 78.2)	67.3 (109/162) (59.8, 74.0)
HPV-negative clinical sample 1	99.1 (107/108) (94.9, 99.8)	100 (162/162) (97.7, 100)
HPV-negative clinical sample 2	97.2 (105/108) (92.1, 99.1)	100 (162/162) (97.7, 100)
PreservCyt Solution 1	99.1 (107/108) (94.9, 99.8)	100 (162/162) (97.7, 100)
PreservCyt Solution 2	99.1 (107/108) (94.9, 99.8)	100 (161/161) (97.7, 100)

IVT = in vitro transcript.
*Expected % negative agreement > 75% and < 100%.

Table 61: 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 62: 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 sample 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 37) in the Tjgris 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 38) in the Tigris DTS System section for results.

Bibliography

1. **Walboomers, J. M., M.V. Jacobs, M.M. Manos, F.X. Bosch, J.A. Kummer, K.V. Shah, P.J. Snijders, J. Peto, C. J. Meijer, N. Muñoz.** 1999. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. **189**:12-19.
2. **Li N., S. Franceschi, R. Howell-Jones, P. J. Snijders, G. M. Clifford.** 2010. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. Int J Cancer, n/a. doi: 10.1002/ijc.25396.
3. **Czegledy J., C. Losif, B.G. Hansson, M. Evander, L. Gergely, and G. Wadell**. 1995. Can a test for E6/E7 transcripts of human papillomavirus type 16 serve as a diagnostic tool for the detection of micrometastasis in cervical cancer? Int J Cancer. **64(3)**:211-5.
4. **Doorbar, J**. 2006. Molecular biology of human papillomavirus infection and cervical cancer. Clin Sci (Lond). **110(5)**:525-41.
5. **Burd, E.M.** 2003. Human papillomavirus and cervical cancer. Clin Microbiol Rev. **16(1)**:1-17.
6. **Lambert P.F., H. Pan, H.C. Pitot, A. Liem, M. Jackson, and A.E. Griep**. 1993. Epidermal cancer associated with expression of human papillomavirus type 16 E6 and E7 oncogenes in the skin of transgenic mice. Proc Natl Acad Sci U S A. **90(12)**:5583-7.
7. **Kjaer S.K., A.J.C. van den Brule, G., Paull, E.I. Svare, M.E. Sherman, B.L. Thomsen, M. Suntum, J.E. Bock, P.A. Poll, and C.J.L.M. Meijer.** 2002. Type specific persistence of high risk human papillomavirus (HPV) as indicator of high grade cervical squamous intraepithelial lesions in young women: population based prospective follow up study. BMJ. **325(7364)**: 572-579.
8. **Monsonego J., F.X. Bosch, P. Coursaget, J.T. Cox, E. Franco, I. Frazer, R. Sankaranarayanan, J. Schiller, A. Singer, T.C. Wright Jr, W. Kinney, C.J. Meijer, J. Linder, E. McGoogan, and C. Meijer**. 2004. Cervical cancer control, priorities and new directions. Int J Cancer. **108(3)**:329-33. Erratum in: Int J Cancer. **108(6)**:945.
9. **Cuschieri, K.S., M.J. Whitley, H.A. Cubie.** 2004. Human papillomavirus type specific DNA and RNA persistence--implications for cervical disease progression and monitoring. J. Med. Virol. **73(1)**: 65-70.
10. **Baseman J.G., and L.A. Koutsky**. 2005. The epidemiology of human papillomavirus infections. J Clin Virol. **32 Suppl 1**:S16-24.
11. **Wu R, Belinson SE, Du H, Na W, Qu X, Wu R, et al.** Human papillomavirus messenger RNA assay for cervical cancer screening: the Shenzhen Cervical Cancer Screening Trial I. International Journal of Gynecological Cancer: official journal of the International Gynecological Cancer Society. 2010; 20(8):1411-4.
12. **Ratnam S, Coutlee F, Fontaine D, Bentley J, Escott N, Ghatage P, et al.** Aptima HPV E6/E7 mRNA test is as sensitive as Hybrid Capture 2 Assay but more specific at detecting cervical precancer and cancer. Journal of Clinical Microbiology. 2011; 49(2):557-64.
13. **Monsonego J, Hudgens MG, Zerat L, Zerat J-C, Syrjänen K, Halfon P, et al.** Evaluation of oncogenic human papillomavirus RNA and DNA tests with liquid-based cytology in primary cervical cancer screening: the FASE study. International Journal of Cancer Journal international du cancer. 2011;129:691-701.
14. **Monsonego J, Hudgens MG, Zerat L, Zerat J-C, Syrjänen K, Smith JS.** Risk assessment and clinical impact of liquid-based cytology, oncogenic human papillomavirus (HPV) DNA and mRNA testing in primary cervical cancer screening (the FASE study). Gynecologic Oncology. 2012;125:175-80.
15. **Nieves L, Enerson CL, Belinson S, Brainard J, Chiesa-Vottero A, Nagore N, et al.** Primary cervical cancer screening and triage using an mRNA human papillomavirus assay and visual inspection. International Journal of Gynecological Cancer: official journal of the International Gynecological Cancer Society. 2013;23(3):513-8.
16. **Cuzick J, Cadman L, Mesher D, Austin J, Ashdown-Barr L, Ho L, et al.** Comparing the performance of six human papillomavirus tests in a screening population. British Journal of Cancer. 2013;108:908-13.
17. **Rebolj M, Preisler S, Ejegod DM, Bonde J, Rygaard C, Lynge E.** Prevalence of human papillomavirus infection in unselected SurePath samples using the APTIMA HPV mRNA assay. The Journal of Molecular Diagnostics:2013;15(5):670-7.
18. **Rebolj M, Bonde J, Ejegod D, Preisler S, Rygaard C, Lynge E.** A daunting challenge: human papillomavirus assays and cytology in primary cervical screening of women below age 30 years. European Journal of Cancer. 2015;51:1456-66.
19. **Heideman DAM, Hesselink AT, van Kemenade FJ, Iftner T, Berkhof J, Topal F, et al.** The Aptima HPV assay fulfills the cross-sectional clinical and reproducibility criteria of international guidelines for human papillomavirus test requirements for cervical screening. Journal of Clinical Microbiology. 2013;51(11):3653-7.
20. **Pyne MT, Hamula CL, Tardif K, Law C, Schlaberg R.** High-risk HPV detection and genotyping by APTIMA HPV using cervical samples. Journal of Virological Methods. 2015;221:95-9.
21. **Iftner T, Becker S, Neis KJ, Castanon A, Iftner A, Holz B, et al.** Head-to-Head Comparison of the RNA368 Based Aptima Human Papillomavirus (HPV) Assay and the DNA-Based Hybrid Capture 2 HPV Test in a Routine Screening Population of Women Aged 30 to 60 Years in Germany. Journal of Clinical Microbiology. 2015;53:2509-16.
22. **Rebolj M, Bonde J, Preisler S, Ejegod D, Rygaard C, Lynge E.** Human Papillomavirus Assays and Cytology in Primary Cervical Screening of Women Aged 30 Years and Above. PLoS One. 2016 Jan 20;11(1):e0147326.
23. **Rebolj M, Bonde J, Ejegod D, Preisler S, Rygaard C, Lynge E.** A daunting challenge: Human Papillomavirus assays and cytology in primary cervical screening of women below age 30years. Eur J Cancer. 2015 Jul;51(11):1456-66.
24. **Kacian, D.L. and T.J. Fultz.** 1995. Nucleic acid sequence amplification methods. U. S. Patent 5,399,491.
25. **Arnold, L. J., P. W. Hammond, W. A. Wiese, and N. C. Nelson.** 1989. Assay formats involving acridinium-ester-labeled DNA probes. Clin Chem. **35**: 1588-1594.
26. **Nelson, N. C., A. BenCheikh, E. Matsuda, and M. Becker.** 1996. Simultaneous detection of multiple nucleic acid targets in a homogeneous format. Biochem. **35**:8429-8438.

27. **Clad, A., M. Reuschenbach, J. Weinschenk, R. Grote, J. Rahmsdorf, and N. Freudenberg.** Performance of the Aptima high-risk HPV mRNA assay in a referral population in comparison with Hybrid Capture 2 and cytology. 2010. J Clin Microbiol, n/a. doi: 10.1128/JCM.01674-10.
28. **Ratnam S., F. Coutless, D. Fontaine, J. Bentley, N. Escott, P. Ghatage, G. Holloway, E. Bartellas, N. Kum, and A. Lear.** 2008. Clinical Correlations of Aptima HPV E6/E7 mRNA Test in Cervical Cancer Screening: Preliminary Results from a Multicentre Candian Study. Presented at EUROGIN 2008, November 12-15, 2008, Scientific Communication SS **8-6.**
29. **Szarewski A., L. Ambroisine, L. Cadman, J. Austin, L. Ho, G. Terry, S. Little, R. Dina, J. McCarthy, H. Buckley, C. Bergeron, P. Soutter, D. Lyons, and J. Cuzick.** 2008. Comparison of predictors for High-Grade Cervical Intraepithelian Neoplasia in Women with Abnormal Smears. Cancer Epidemiol Biomarkers Prev. **17(11)**, November.
30. **Castle P.E., J. Dockter, C. Giachetti, F.A.R. Garcia, M. McCormick, A.L.Mitchell, E.B. Holladay, and D.P. Kolk.** 2007. A Cross-sectional Study of a Prototype Carcinogenic Human Papillomavirus E6/E7Messenger RNA Assay for Detection of Cervical Pre-cancer and Cancer. Clin Cancer Res. **13(9)**. 2599.
31. **Monsonego J., M.G. Hudgens, L. Zerat, J.C. Zerat, K. Syrjänen, P. Halfon, F. Ruiz, and J.S. Smith.** 2010. Evaluation of oncogenic human papillomavirus RNA and DNA tests with liquid based cytology in primary cervical cancer screening (The FASE study). Int J Cancer**.** n/a. doi 10.1002/ljc.25726.
32. **Datta, S. D., L. A. Koutsky, S. Ratelle, E. R. Unger, J. Shlay, T. McClain, B. Weaver, P. Kerndt, J. Zenilman, M. Hagensee, C. J. Suhr, and H. Weinstock.** 2008. Human Papillomavirus Infection and Cervical Cytology in Women Screened for Cervical Cancer in the United States, 2003–2005. Annals Int Med. **148**:493.
33. **Clifford, G.M., S. Gallus, R. Herrero, N. Muñoz, P. J. F. Snijders, S. Vaccarella, P. T. H. Anh, C. Ferreccio, N. T. Hieu, E. Matos, M. Molano, R. Rajkumar, G. Ronco, S. de Sanjosé, H. R. Shin, S. Sukvirach, J. O. Thomas, S. Tunsakul, C. J. L. M. Meijer, S. Franceschi, and the IARC HPV Prevalence Surveys Study Group.** Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled Analysis. 2005. The Lancet. **366**, 991.
34. **Stoler, M.H., T.C. Wright, Jr., J. Cuzick, J. Dockter, J. Reid, D. Getman, C. Giachetti.** 2013. Aptima HPV assay performance in women with atypical squamous cells of undetermined significance cytology results. American Journal of Obstetrics & Gynecology. **208(2)**:144-145.
35. **Wright TC, Jr., Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, and Solomon D.** 2006 Consensus Guidelines for the Management of Women with Abnormal Cervical Cancer Screening Tests. 2007. Am J Obstet Gynecol 197 (4); 346-355.
36. **Pretorius R.G., W. H. Zhang, J. L. Belinson, et al.** Colposcopically directed biopsy, random cervical biopsy, and endocervical curettage in the diagnosis of cervical intraepithelial neoplasia II or worse. 2004. Am J Obstet Gynecol. **191**:430-434.
37. **Pretorius R.G., R. J. Kim, J. L. Belinson, P. Elson, Y-L Qiao.** Inflation of sensitivity of cervical cancer screening tests secondary to correlated error in colposcopy. 2006. J Low Genit Tract Dis. **10(1)**:5-9.



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