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# Aptima® HPV Assay (Panther® System)

Instructions for Use
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.

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

## 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.[1, 4]

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

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

### 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. At least 25 peer-reviewed publications[11-35] from 15 separate clinical studies report the performance of Aptima HPV in primary screening in women enrolled in eleven countries (China, Canada, France, Mexico, England, Denmark, The Netherlands, The United States, Germany, Sweden, and Thailand). 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®),[42] and detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA).[43] 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.[44] 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.[44]

## Summary of Safety and Performance

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

## Warnings and Precautions

A. For *in vitro* diagnostic use.

B. For professional use.

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

### Laboratory Related

D. Use only supplied or specified disposable laboratory ware.

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

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

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

### Specimen Related

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

I. Expiration dates listed on specimen collection/transfer kits and tubes pertain to the 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 *Panther 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 and Aptima Auto Detect Reagents are not part of the Master Lot; any lot may be used.

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

U. Tips with hydrophobic plugs must be used.

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

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

| EU Hazard Information | |
|---|---|
|  | **Selection Reagent**<br>***BORIC ACID 1 – 5%***<br><br>**WARNING**<br>H315 – Causes skin irritation<br>H319 – Causes serious eye irritation |
| — | **Target Capture Reagent**<br>***HEPES 5 – 10%***<br>***EDTA 1 – 5%***<br>***Lithium Hydroxide, Monohydrate 1 – 5%***<br><br>—<br>H412 – Harmful to aquatic life with long lasting effects<br>P273 – Avoid release to the environment<br>P280 – Wear eye protection/ face protection |

| | |
|---|---|
| | **Amplification Reagent**<br>***HEPES 25 - 30%*** |
| — | —<br>H412 - Harmful to aquatic life with long lasting effects<br>P273 - Avoid release to the environment<br>P280 - Wear eye protection/ face protection |
| | **Enzyme Reagent**<br>***HEPES 1 - 5%*** |
| — | —<br>H412 - Harmful to aquatic life with long lasting effects.<br>P273 - Avoid release to the environment<br>P280 - Wear eye protection/ face protection. |
| | **Probe Reagent**<br>***LAURYL SULFATE LITHIUM SALT 35 - 40%***<br>***SUCCINIC ACID 10 - 15%***<br>***LITHIUM HYDROXIDE MONOHYDRATE 10 - 15%*** |
| — | —<br>H412 - Harmful to aquatic life with long lasting effects<br>P273 - Avoid release to the environment<br>P280 - Wear eye protection/ face protection |

## Reagent Storage and Handling Requirements

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

A. The following reagents are stored at 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

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

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 72 hours when stored on board the Panther System.

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

H. **Do not freeze reagents**.

## Specimen Collection and Storage

A. Specimen collection and processing

*ThinPrep liquid cytology specimens*

1. Collect cervical specimens in ThinPrep Pap test vials containing PreservCyt solution with broom-type or cytobrush/spatula collection devices according to the manufacturer's instructions.
2. Prior to or after processing with the ThinPrep 2000 processor,ThinPrep 5000 Processor, ThinPrep 5000 processor with autoloader, or ThinPrep Genesis™ processor, transfer 1 mL of the ThinPrep liquid cytology specimen into an Aptima Specimen Transfer tube according to the instructions in the Aptima Specimen Transfer Kit and Aptima Transfer Solution package insert.

*SurePath liquid cytology specimens*

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

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

***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

   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 and Aptima Transfer Solution 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.*

# 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**<br>*Non-infectious nucleic acids dried in buffered solution containing < 5% bulking agent.* | 1 vial |
| **E** | **HPV Enzyme Reagent**<br>*Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.* | 1 vial |
| **P** | **HPV Probe Reagent**<br>*Non-infectious chemiluminescent DNA probes (< 500 ng/vial) dried in succinate buffered solution containing < 5% detergent.* | 1 vial |
| **IC** | **HPV Internal Control Reagent**<br>*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**<br>*Aqueous solution containing preservatives.* | 1 |
| **ER** | **HPV Enzyme Reconstitution Solution**<br>*HEPES buffered solution containing a surfactant and glycerol.* | 1 |
| **PR** | **HPV Probe Reconstitution Solution**<br>*Succinate buffered solution containing < 5% detergent.* | 1 |
| **S** | **HPV Selection Reagent**<br>*600 mM borate buffered solution containing surfactant.* | 1 |
| **TCR** | **HPV Target Capture Reagent**<br>*Buffered solution containing solid-phase and capture oligomers (< 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**<br>*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**<br>*Buffered solution containing < 5% detergent.* | 5 vials |

## Materials Required But Available Separately

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

| Material | Cat. No. |
|---|---|
| Panther System | 303095 |
| Panther System Continuous Fluid and Waste (Panther Plus) | PRD-06067 |
| Panther Run Kit | 303096 |
| *Aptima Assay Fluids Kit (Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)* | *303014* |
| *Aptima Auto Detect Kit* | *303013* |
| *Multi-tube units (MTUs)* | *104772-02* |
| *Panther Waste Bag Kit* | *902731* |
| *Panther Waste Bin Cover* | *504405* |
| Tips, 1000 µL filtered, conductive, liquid sensing and disposable<br>*Not all products are available in all regions. Contact your representative for region-specific information* | 901121 (10612513 Tecan)<br>903031 (10612513 Tecan)<br>MME-04134 (30180117 Tecan)<br>MME-04128 |
| Aptima Specimen Transfer Kit | 301154C |
| Aptima Specimen Transfer Kit — printable | PRD-05110 |
| Aptima Cervical Specimen Collection and Transport Kit | 302657 |
| Aptima Penetrable Caps | 105668 |
| Replacement non-penetrable caps | 103036A |
| Spare Caps for 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 5.0% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution | — |
| Disposable gloves | — |
| Aptima Transfer Solution Kit (for SurePath specimens only) | 303658 |

## Optional Materials

| Material | Cat. No. |
|---|---|
| Bleach Enhancer for Cleaning | 302101 |

## Panther System Test Procedure

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

A. Work Area Preparation

Clean work surfaces where reagents and samples will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35 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 1, Step 1).
   d. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.
   e. While holding the solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle (Figure 1, Step 2).
   f. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 1, Step 3).
   g. Gently swirl the solution in the bottle to mix thoroughly. Avoid creating foam while swirling the bottle (Figure 1, Step 4).
   h. Wait for the lyophilized reagent to go into solution, then invert the assembled bottles again, tilting at a 45° angle to minimize foaming (Figure 1, Step 5). Allow all of the liquid to drain back into the plastic bottle.
   i. Remove the reconstitution collar and glass vial (Figure 1, Step 6).
   j. Recap the plastic bottle. Record operator initials and the reconstitution date on all reconstituted reagent vials (Figure 1, Step 7).
   k. Discard the reconstitution collar and vial (Figure 1, Step 8).

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

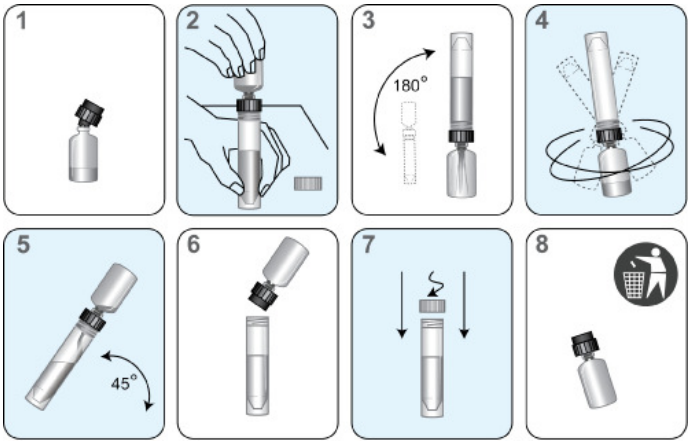


***Figure 1. Panther System Reconstitution Process***

2. Prepare the working Target Capture Reagent (wTCR):
   a. Pair the appropriate bottles of TCR and IC.
   b. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.
   c. Open the bottle of TCR, and set the cap on a clean, covered work surface.
   d. Open the bottle of IC and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the IC bottle.
   e. Cap the bottle of TCR and gently swirl the solution to mix the contents. Avoid creating foam during this step.
   f. Record operator initials and the current date on the label.
   g. Discard the IC bottle and cap.
   h. Precipitate may form in wTCR which may yield invalid results due to volume verification errors. Precipitate may be dissolved by warming wTCR at 42°C to 60°C for up to 90 minutes. Allow the wTCR to equilibrate to room temperature prior to use. Do not use if precipitate persists.
3. Prepare the Selection Reagent
   a. Check the reagent lot number on the Master Lot Barcode Sheet to make sure it belongs to the kit.
   b. If 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 samples**.
3. Inspect sample tubes before loading into the rack. If a sample tube contains bubbles or has a lower volume than is typically observed, centrifuge the tube for 5 minutes at 420 RCF to ensure that there is no liquid in the cap.

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

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.

***IC Cutoff =*** *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

***Analyte Cutoff =*** *[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.

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

# 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*<br>*IC ≥ IC Cutoff*<br>*IC ≤ 2,000,000 RLU* |
| **Positive** | *Analyte S/CO ≥ 0.50*<br>*IC ≤ 2,000,000 RLU*<br>*Analyte ≤ 13,000,000 RLU* |
| **Invalid** | *IC > 2,000,000 RLU*<br>*or*<br>*Analyte S/CO < 0.50 and IC < IC Cutoff*<br>*or*<br>*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 1 mL 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 Panther System has been determined in a non-clinical study to be 0.7%.

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.

# 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.[36,37] 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. 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 1 for the ASC-US (atypical squamous cells of undetermined significance) and the NILM (negative for intraepithelial lesion or malignancy) populations.

**Table 1:** High-risk HPV mRNA Prevalence 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.[38]

The Aptima HPV assay was first launched on the Tigris® DTS system in 2008. In 2011, indications were expanded to use the Aptima HPV assay on the Panther System. The Panther System is an alternative, smaller instrument platform to the Tigris DTS System. Both systems are intended to fully automate amplified nucleic acid testing of diagnostic assays. Select assay performance testing completed on the Tigris DTS System was leveraged to support assay performance on the Panther System.

## 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.[39]

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 2.

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

| Aptima HPV Assay Result* | HPV DNA Test | Consensus Histology Review Panel Diagnosis | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Undetermined** | Normal | CIN1 | CIN2 | CIN3 | Cancer | Total |
| **Positive** | Positive | 6 | 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 3, as are the estimates for the commercially available HPV DNA test.

**Table 3:** 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=937 | | HPV DNA Test N=863* | |
|---|---|---|---|---|---|
| | **Performance** | **Estimate** | **(95% CI)** | **Estimate** | **(95% CI)** |
| **≥CIN2** | **All Biopsies** | | | | |
| | **Sensitivity (%)** | 84.6 (77/91) | (75.8, 90.6) | 88.8 (79/89) | (80.5, 93.8) |
| | **Specificity (%)** | 62.1 (525/846) | (58.7, 65.3) | 55.8 (432/774) | (52.3, 59.3) |
| | **PPV (%)** | 19.3 (77/398) | (17.3, 21.2) | 18.8 (79/421) | (17.0, 20.4) |
| | **NPV (%)** | 97.4 (525/539) | (96.0, 98.5) | 97.7 (432/442) | (96.2, 98.8) |
| | **Prevalence (%)** | 9.7 (91/937) | | 10.3 (89/863) | |
| | **Directed Biopsies**** | | | | |
| | **Sensitivity (%)** | 90.0 (54/60) | (79.9, 95.3) | 93.2 (55/59) | (83.8, 97.3) |
| | **Specificity (%)** | 60.8 (531/874) | (57.5, 63.9) | 54.5 (437/802) | (51.0, 57.9) |
| | **PPV (%)** | 13.6 (54/397) | (12.0, 15.0) | 13.1 (55/420) | (11.7, 14.2) |
| | **NPV (%)** | 98.9 (531/537) | (97.8, 99.6) | 99.1 (437/441) | (97.9, 99.7) |
| | **Prevalence (%)** | 6.4 (60/934) | | 6.9 (59/861) | |
| **≥CIN3** | **All Biopsies** | | | | |
| | **Sensitivity (%)** | 90.2 (37/41) | (77.5, 96.1) | 92.3 (36/39) | (79.7, 97.3) |
| | **Specificity (%)** | 59.7 (535/896) | (56.5, 62.9) | 53.3 (439/824) | (49.9, 56.7) |
| | **PPV (%)** | 9.3 (37/398) | (8.0, 10.3) | 8.6 (36/421) | (7.4, 9.4) |
| | **NPV (%)** | 99.3 (535/539) | (98.3, 99.8) | 99.3 (439/442) | (98.3, 99.8) |
| | **Prevalence (%)** | 4.4 (41/937) | | 4.5 (39/863) | |
| | **Directed Biopsies**** | | | | |
| | **Sensitivity (%)** | 93.1 (27/29) | (78.0, 98.1) | 96.4 (27/28) | (82.3, 99.4) |
| | **Specificity (%)** | 59.1 (535/906) | (55.8, 62.2) | 52.8 (440/834) | (49.4, 56.1) |
| | **PPV (%)** | 6.8 (27/398) | (5.7, 7.5) | 6.4 (27/421) | (5.5, 7.0) |
| | **NPV (%)** | 99.6 (535/537) | (98.8, 100) | 99.8 (440/441) | (98.9, 100) |
| | **Prevalence (%)** | 3.1 (29/935) | | 3.2 (28/862) | |

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

When evaluating all biopsies, clinical sensitivity estimates of the Aptima HPV assay and the 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.[40,41] 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 4 and Table 5 (≥CIN2 and ≥CIN3, respectively, based on evaluating all biopsies).

**Table 4:** ASC-US ≥ 21 Years Population: Performance of the Aptima HPV Assay and an 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 5:** 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 6, 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 6:** 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 7.

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

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

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

## NILM ≥ 30 Years Population: Aptima HPV Assay Clinical Performance 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 8.

**Table 8:** 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 9). 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 9:** 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 10. 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 11 and by age group in Table 12.

**Table 10:** NILM ≥ 30 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an 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 11:** 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 12:** 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

| | | | Aptima HPV Assay N=818 | | HPV DNA Test N=800* | |
|---|---|---|---|---|---|---|
| | **Age** | **Assay Result** | **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 13, as are the estimates for the commercially available HPV DNA test. Unadjusted clinical performance estimates are shown in Table 14. 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 13:** 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 14:** 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 15 and Table 16, respectively).

**Table 15:** 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 16:** 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 17.

**Table 17:** 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 18.

**Table 18:** NILM ≥ 30 Years Population: 3-Year Cumulative Absolute and Relative Risks* of ≥CIN2 and ≥CIN3 for Results of the Aptima HPV Assay and an 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=1350) 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 19, with the positive and negative predictive values.

**Table 19:** Performance of the Aptima HPV Assay and an HPV PCR Test for Detection of ≥CIN3

| Performance | Aptima HPV Assay N=1326 | | HPV PCR Test N=1351 | |
|---|---|---|---|---|
| | Estimate | (95% CI) | Estimate | (95% CI) |
| **Sensitivity (%)** | 89.6 (69/77) | (80.8 - 94.6) | 94.8 (73/77) | (87.4 - 98.0) |
| **Specificity (%)** | 57.3 (716/1249) | (54.6 - 60.0) | 52.0 (663/1274) | (49.3 - 54.8) |
| **PPV (%)** | 11.5 (69/602) | (10.3 - 12.4) | 10.7 (73/684) | (9.8 - 11.4) |
| **NPV (%)** | 98.9 (716/724) | (98.0 - 99.5) | 99.4 (663/667) | (98.6 - 99.8) |
| **Prevalence (%)** | 5.8 (77/1326) | | 5.7 (77/1351) | |

## 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 20.

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 20:** 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

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 21.

For CSCT specimens, overall agreement between the Panther and Tigris DTS Systems was > 98%, as shown in Table 21. 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 21:** 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 22 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 22:** 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 23a (panel members with expected positive results) and Table 23b (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 24 for Study 1 and Table 25 for Study 2.

**Table 23a:** Aptima HPV Assay Precision Study 1 and 2: Panel Description, Positive Agreement, and Percentile Distribution of Analyte S/CO Values for Panel Members with Expected Positive Results

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

IVT = in vitro transcript

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

**Table 23b:** Aptima HPV Assay Precision Study 1 and 2: Panel Description, Negative Agreement, and Percentile Distribution of Analyte S/CO Values for Panel Members with Expected Negative Results

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

**Table 24:** 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 |

CV = coefficient of variation; IVT = in vitro transcript; SD = standard deviation

*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)).

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

**Table 25:** Aptima HPV 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 |

CV = coefficient of variation; IVT = in vitro transcript; SD = standard deviation
*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)).

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

## Cross-Reactivity

***Note:*** *Testing with potentially cross-reactive organisms for the Aptima HPV assay was performed using the Tigris DTS System. The Aptima HPV assay was first launched on the Tigris DTS System in 2008. In 2011, indications were expanded to use the Aptima HPV assay on the Panther System. The Panther System is an alternative, smaller instrument platform to the Tigris DTS System. Both systems are intended to fully automate amplified nucleic acid testing of diagnostic assays. Select assay performance testing completed on the Tigris DTS System was leveraged to support assay performance on the Panther System.*

The analytical specificity of the Aptima HPV assay was evaluated with PreservCyt solution media diluted 1:2.9 into STM and spiked with cultured bacteria, yeast, or fungi; cultured virus; or low-risk HPV *in vitro* transcripts. The organisms and test concentrations are identified in Table 26. The study criteria for assessing the effect of the presence of

microorganism on the specificity of the assay were based on positivity. Cross-reactivity was observed with low-risk HPV genotypes 26, 67, 70, and 82, but not with any of the other organisms tested.

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

| Organism | Test Concentration with No Cross-Reactivity | Organism | Test Concentration with No Cross-Reactivity |
|---|---|---|---|
| **Bacteria** | | | |
| *Acinetobacter lwoffii* | $1x10^8$ CFU/mL | *Listeria monocytogenes* | $1x10^8$ CFU/mL |
| *Actinomyces israelii* | $1x10^8$ CFU/mL | *Micrococcus luteus* | $1x10^8$ CFU/mL |
| *Alcaligenes faecalis* | $1x10^8$ CFU/mL | *Mobiluncus curtisii* | $2x10^7$ CFU/mL |
| *Atopobium vaginae* | $5x10^7$ CFU/mL | *Mycobacterium smegmatis* | $1x10^8$ CFU/mL |
| *Bacillus cereus* | $1x10^8$ CFU/mL | *Mycoplasma fermentans* | $5x10^7$ CFU/mL |
| *Bacteroides fragilis* | $1x10^8$ CFU/mL | *Mycoplasma genitalium* | $1x10^8$ CFU/mL |
| *Bacteroides ureolyticus* | $1x10^8$ CFU/mL | *Mycoplasma hominis* | $5x10^7$ CFU/mL |
| *Bifidobacterium adolescentis* | $1x10^8$ CFU/mL | *Neisseria gonorrhoeae* | $1x10^8$ CFU/mL |
| *Bifidobacterium breve* | $1x10^8$ CFU/mL | *Neisseria gonorrhoeae and Chlamydia trachomatis* | $2.5x10^7$ CFU/mL<br>$2.3x10^5$ $TCID_{50}$/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 | | |
| **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 |

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

| Organism | Test Concentration with No Cross-Reactivity | Organism | Test Concentration with No Cross-Reactivity |
|---|---|---|---|
| Epstein-Barr virus | $4.3x10^6$ vp/mL | SV40 | $1.2 x10^4$ $TCID_{50}$/mL |
| HIV-1 | $1.0x10^6$ copies/mL | | |
| **Non-targeted HPV genotypes** | | | |
| HPV 6 | $2.5x10^6$ copies/mL | HPV 61 | $2.5x10^6$ copies/mL |
| HPV 11 | $2.5x10^6$ copies/mL | **HPV 67** | 1 copy/mL |
| **HPV 26** | 2.5 copies/mL | HPV 69 | $2.5x10^6$ copies/mL |
| HPV 30 | $2.5x10^6$ copies/mL | **HPV 70** | 1 copy/mL |
| HPV 34 | $2.5x10^6$ copies/mL | HPV 71 | $2.5x10^6$ copies/mL |
| HPV 42 | $2.5x10^6$ copies/mL | HPV 73 | $2.5x10^6$ copies/mL |
| HPV 43 | $2.5x10^6$ copies/mL | HPV 81 | $2.5x10^6$ copies/mL |
| HPV 44 | $2.5x10^6$ copies/mL | **HPV 82** | 1 copy/mL |
| HPV 53 | $2.5x10^6$ copies/mL | HPV 85 | $2.5x10^6$ copies/mL |
| HPV 54 | $2.5x10^6$ copies/mL | | |

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

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

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

## Interference

***Note:*** *Testing with potential interfering substances for the Aptima HPV assay was performed using the Tigris DTS System. The Aptima HPV assay was first launched on the Tigris DTS System in 2008. In 2011, indications were expanded to use the Aptima HPV assay on the Panther System. The Panther System is an alternative, smaller instrument platform compared to the Tigris DTS System. Both systems are intended to fully automate amplified nucleic acid testing of diagnostic assays. Select assay performance testing completed on the Tigris DTS System was leveraged to support assay performance on the Panther System.*

The substances described in Table 27 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 the other substances tested.

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

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

*Personal lubricants that contain Polyquaternium 15.

## Pre- and Post-cytology ThinPrep Liquid Cytology Samples Processed on ThinPrep 2000 Processor

Testing was conducted to demonstrate the equivalence of ThinPrep liquid Pap clinical specimens with aliquots removed before and after processing on the ThinPrep 2000 processor. Fifty (50) pre- and post-processed sample pairs were tested with each of the three reagent lots for a total of 150 sample sets. Overall agreement between the pre- and post-processed samples was 96.0% (CI 95%: 91.6% - 98.2%). The positive agreement (using post-processed samples as the reference) was 95.6% (CI 95%: 89.2% - 98.3%) and negative agreement was 96.6% (CI 95%: 88.5% - 99.1%). The kappa coefficient was 0.92.

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

Testing was conducted to determine the agreement of ThinPrep liquid cytology samples in PreservCyt Solution tested on the Aptima HPV assay before and after processing on the ThinPrep 5000 Processor. A total of 200 contrived ThinPrep liquid cytology samples (100 HPV positive, 100 HPV negative) were evaluated in the Aptima HPV assay before and after processing on the ThinPrep 5000 Processor. The study showed comparable performance between pre- and post-cytology samples at all concentrations tested (Table 28).

**Table 28:** Pre- and Post-Cytology Sample Results

| | | Pre-cytology | | | |
|---|---|---|---|---|---|
| | | Positive Samples (above C95) | | Negative Samples (below C95) | |
| | | Spiked with HeLa at ~10X LoD (95% CI) | Spiked with HeLa at 1.5-3X LoD (95% CI) | Spiked with HeLa at 0.05X LoD (95% CI) | Un-spiked (95% CI) |
| Post-cytology | Positive Percent Agreement | 100.0 | 98.7 | 0.0 | N/A |
| | | (83.9, 100.0) | (93.2, 99.8) | (0.0, 79.3) | |
| | | 20/20 | 78/79 | 0/1 | |
| | Negative Percent Agreement | N/A | 0.0 | 97.4 | 100.0 |
| | | | (0.0, 79.3) | (86.8, 99.5) | (94.0, 100.0) |
| | | | 0/1 | 38/39 | 60/60 |
| Total | | 20 | 80 | 40 | 60 |

CI = Confidence Interval

## Pre- and Post-Cytology ThinPrep Liquid Cytology Samples Processed on Genesis Processor

Testing was conducted to demonstrate the equivalence of ThinPrep liquid Pap clinical specimens with aliquots removed before and after processing on the Genesis Processor. From each pre-processing sample, two unique aliquots were tested. For samples where results from both pre-processing aliquots were in agreement, a composite pre-processing reference result was then used to calculate agreement with a post-processing aliquot from the same sample. For 2,068 samples with a composite reference result, the overall agreement between pre-processing and post-processing results was 98.2% (95% CI 97.5-98.7%). The positive agreement was 97.9% (95% CI 94.7-99.2%), and the negative agreement was 98.2% (95% CI: 97.5-98.7%).

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



AW-22202-001 Rev. 002
2024-08

| Revision History | Date | Description |
|---|---|---|
| AW-22202 Rev. 001 | March 2023 | • Created Aptima™ HPV Assay (Panther™ System) assay IFU AW-22202 Rev. 001 based on AW-14517 Rev. 007 for regulatory compliance with IVDR.<br>• Updated the Intended Use by removing reference for use on the Tigris DTS System.<br>• Added Summary of Safety and Performance.<br>• Updated EU Hazard Information.<br>• Updated sections of Warnings and Precautions, Reagent Storage and Handling Requirements, Specimen Collection and Storage, Reagents and Materials Provided, Materials Required But Available Separately, Panther System Test Procedure, Limitations, Assay Precision tables, Cross-Reactivity, Interference, and Bibliography.<br>• Updated contact information including: EC Rep, CE Mark, Australian Rep information, and technical support.<br>• Miscellaneous style and formatting updates. |
| AW-22202 Rev. 002 | August 2024 | • Updated Clinical Performance SurePath Specimens table. |