APTIMA® HPV Assay

For in vitro diagnostic use.
For U.S. Export only.

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General Information

Intended Use

The APTIMA HPV Assay is a target amplification nucleic acid probe test for the \textit{in vitro} qualitative detection of E6/E7 viral messenger RNA (mRNA) from 14 high-risk HPV types (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. Cervical specimens collected in ThinPrep Pap Test vials containing PreservCyt Solution may be tested with the APTIMA HPV Assay either pre- or post-Pap processing, as well as cervical specimens collected with the APTIMA Cervical Specimen Collection and Transport Kit. The assay can be used to test these specimen types with either the Direct Tube Sampling (DTS) Systems or the TIGRIS DTS System. Additionally, the assay can be used to test cervical specimens collected with the APTIMA Cervical Specimen Collection and Transport Kit on the PANTHER System.

Summary and Explanation of the Test

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

Only 14 of the genotypes are considered pathogenic or high-risk for cervical disease.\textsuperscript{4} Multiple studies have linked genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 to disease progression.\textsuperscript{2,5,6} Patients with a persistent infection with one of these types have an increased risk for developing severe dysplasia or cervical carcinoma.\textsuperscript{4,7} HPV types 6, 11, 42, 43, and 44, which cause genital warts, are considered low-risk types or non-pathogenic with respect to cervical cancer.\textsuperscript{8}

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 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.\textsuperscript{1,8}

Currently, clinical detection of HPV is typically performed by \textit{in vitro} diagnostic assays that detect viral genomic DNA, specifically the L1 gene. However, because HPV infections are very common and because most women will clear HPV infections within 6 to 12 months,\textsuperscript{2,9} the presence of HPV DNA does not mean that cervical dysplasia or cervical cancer is present. A more efficacious approach for detection of cervical disease is to target those oncogenic elements of HPV that foster persistent viral infection and cellular transformation.\textsuperscript{10}

Principles of the Procedure

The APTIMA HPV Assay involves three main steps, which take place in a single tube: target capture, target amplification by Transcription-Mediated Amplification (TMA),\textsuperscript{11} and detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA).\textsuperscript{12} The assay incorporates an internal control 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 a solution 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 in a luminometer and are reported as Relative Light Units (RLU).

Internal Control is added to each reaction via the Target Capture Reagent. The Internal Control monitors the target capture, amplification, and detection steps of the assay. Internal Control signal in each reaction is discriminated from the HPV signal by the differential kinetics of light emission from probes with different labels. Internal Control 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.

**Warnings and Precautions**

A. For *in vitro* diagnostic use.

For additional specific warnings and precautions refer to the DTS Systems, TIGRIS DTS System, and PANTHER System Operator's Manuals.

**Laboratory Related**

B. Use only supplied or specified disposable laboratory ware.

C. 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.

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

**DTS Systems Specific**

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

**Specimen Related**

G. 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.

H. 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.

I. 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.

J. Upon piercing, liquid can discharge from tube caps under certain conditions. Refer to DTS Systems Test Procedure, TiGRIS DTS System Test Procedure, or PANTHER System Test Procedure for more information.

K. Samples should be rejected if a collection device has been left in the sample tube.

**Assay Related**

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

M. Avoid microbial and ribonuclease contamination of reagents.

N. Do not use kit after its expiration date.

O. Do not interchage, mix, or combine assay reagents from different master lots. APTIMA Assay Fluids, Auto Detect Reagents, and APTIMA HPV Assay Controls are not part of the master lot; any lot may be used.

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

Q. Tips with hydrophobic plugs must be used.
**DTS Systems Specific**

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

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

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

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

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

**Reagent Storage and Handling Requirements**

A. The following reagents are stored at 2°C to 8°C (refrigerated) upon receipt:
   - HPV Amplification Reagent
   - HPV Enzyme Reagent
   - HPV Probe Reagent
   - HPV Internal Control Reagent
   - HPV Calibrators
   - HPV Controls (DTS Systems and TIGRIS DTS System only)

B. The following reagents are stored at 15°C to 30°C:
   - HPV Amplification Reconstitution Solution
   - HPV Enzyme Reconstitution Solution
   - HPV Probe Reconstitution Solution
   - HPV Target Capture Reagent
   - HPV Selection Reagent
   - Wash Solution
   - Oil Reagent
   - Buffer for Deactivation Fluid
   - Auto Detect Reagent 1
   - Auto Detect Reagent 2
   - APTIMA System Fluid Preservative (TIGRIS DTS System only)

C. After reconstitution, the following reagents are stable for 30 days when stored at 2°C to 8°C (refrigerated):
   - 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.
E. Discard any unused reconstituted reagents and wTCR after 30 days or after the Master Lot expiration date, whichever comes first.

F. The APTIMA HPV Assay reagents are stable for a cumulative 48 hours when stored on-board the TIGRIS DTS System.

G. The APTIMA HPV Assay reagents are stable for a cumulative 72 hours when stored on-board the PANTHER System.

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

I. Do not freeze reagents.

**Specimen Collection and Storage**

A. Specimen collection

*PreservCyt liquid Pap specimens (DTS Systems and TIGRIS DTS System only)*

1. Collect a PreservCyt liquid Pap specimen according to the ThinPrep Pap Test instructions for use.
2. Prior to or after processing with the ThinPrep Processor, transfer 1 mL of the PreservCyt liquid Pap specimen into an APTIMA Specimen Transfer Tube. Gently invert the tube 2 to 3 times to ensure complete mixture of the specimen. Avoid splashing and cross-contamination. For further details, refer to the APTIMA Specimen Transfer Kit instructions for use.

*APTIMA Cervical Specimen Collection and Transport Kit specimens*

Collect the specimen according to the APTIMA Cervical Specimen Collection and Transport Kit instructions for use.

B. Transport and storage before testing

*PreservCyt liquid Pap specimens (DTS Systems and TIGRIS DTS System only)*

1. Transport the PreservCyt liquid Pap specimen at 2°C to 30°C.
2. Specimens should be transferred to an APTIMA Specimen Transfer Tubes within 105 days of collection.
3. Prior to transfer, PreservCyt liquid Pap specimens should be stored at 2°C to 8°C, with no more than 30 days at temperatures up to 30°C.
4. PreservCyt liquid Pap 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 PreservCyt liquid Pap specimen or the PreservCyt liquid Pap specimen diluted into the Specimen Transfer Tube may be stored at -20°C or colder for up to 24 months.

*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. 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:** National, regional, and local requirements for packaging must be met when specimens are transported by common land and air carriers.
DTS Systems

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

Reagents and Materials Provided

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

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

**APTIMA HPV Refrigerated Box**
(store at 2°C to 8°C upon receipt)

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>HPV Amplification Reagent</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>Non-infectious nucleic acids dried in buffered solution containing &lt; 5% bulking agent.</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>HPV Enzyme Reagent</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing &lt; 10% bulking reagent.</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>HPV Probe Reagent</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>Non-infectious chemiluminescent DNA probes (&lt; 500 ng/vial) dried in succinate buffered solution containing &lt; 5% detergent.</td>
<td></td>
</tr>
<tr>
<td>IC</td>
<td>HPV Internal Control Reagent</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>RNA Transcript in buffered solution containing &lt; 5% detergent.</td>
<td></td>
</tr>
</tbody>
</table>

**APTIMA HPV Room Temperature Box**
(store at 15°C to 30°C upon receipt)

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR</td>
<td>HPV Amplification Reconstitution Solution</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>Aqueous solution containing preservatives.</td>
<td></td>
</tr>
<tr>
<td>ER</td>
<td>HPV Enzyme Reconstitution Solution</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>HEPES buffered solution containing a surfactant and glycerol.</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>HPV Probe Reconstitution Solution</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>Succinate buffered solution containing &lt; 5% detergent.</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>HPV Selection Reagent</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>600 mM borate buffered solution containing surfactant.</td>
<td></td>
</tr>
<tr>
<td>TCR</td>
<td>HPV Target Capture Reagent</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>Non-infectious nucleic acid in a buffered solution containing solid phase (&lt; 0.5 mg/mL).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sealing Cards</td>
<td>1 package</td>
</tr>
<tr>
<td></td>
<td>Reconstitution Collars</td>
<td>3</td>
</tr>
</tbody>
</table>
APTIMA HPV Calibrators Box (Cat. No. 302554)  
(store at 2°C to 8°C upon receipt)

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCAL</td>
<td>HPV Positive Calibrator</td>
<td>5 vials</td>
</tr>
<tr>
<td></td>
<td><em>Non-infectious HPV nucleic acid in a buffered solution containing &lt; 5% detergent.</em></td>
<td></td>
</tr>
<tr>
<td>NCAL</td>
<td>HPV Negative Calibrator</td>
<td>5 vials</td>
</tr>
<tr>
<td></td>
<td><em>Buffered solution containing &lt; 5% detergent.</em></td>
<td></td>
</tr>
</tbody>
</table>

APTIMA HPV Controls Box (Cat. No. 302556)  
(store at 2°C to 8°C upon receipt)

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>HPV Positive Control</td>
<td>5 vials</td>
</tr>
<tr>
<td></td>
<td><em>Lysed, inactivated HPV Positive cultured cells in a buffered solution containing &lt; 5% detergent.</em></td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>HPV Negative Control</td>
<td>5 vials</td>
</tr>
<tr>
<td></td>
<td><em>Lysed, inactivated HPV Negative cultured cells in a buffered solution containing &lt; 5% detergent.</em></td>
<td></td>
</tr>
</tbody>
</table>

Materials Required But Available Separately

*Note: Materials available from Gen-Probe have catalog numbers listed, unless otherwise specified.*

<table>
<thead>
<tr>
<th>Component</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEADER HC+ Luminometer</td>
<td>104747</td>
</tr>
<tr>
<td>GEN-PROBE Target Capture System (TCS)</td>
<td>104555</td>
</tr>
<tr>
<td>2 SB100 Dry Heat Bath/Vortexers</td>
<td>105524F</td>
</tr>
<tr>
<td>APTIMA Auto Detect Kit</td>
<td>301048C</td>
</tr>
<tr>
<td>APTIMA Assay Fluids Kit</td>
<td>302002C</td>
</tr>
<tr>
<td>Micropipettor, 1000 µL RAININ PR1000</td>
<td>104216</td>
</tr>
<tr>
<td>2 eppendorf Repeater Plus Pipettors</td>
<td>105725</td>
</tr>
<tr>
<td>Repeat pipettor tips (2.5 mL, 5.0 mL, 25.0 mL)</td>
<td>—</td>
</tr>
<tr>
<td>Tips, 1000 µL P1000</td>
<td>105049</td>
</tr>
<tr>
<td>Ten Tube Units (TTU)</td>
<td>TU0022</td>
</tr>
<tr>
<td>TTU rack</td>
<td>104579</td>
</tr>
<tr>
<td>Ten Tip Cassettes (TTC)</td>
<td>104578</td>
</tr>
<tr>
<td>APTIMA Specimen Transfer Kit</td>
<td>301154C</td>
</tr>
<tr>
<td>APTIMA Cervical Specimen Collection and Transport Kit</td>
<td>302657</td>
</tr>
<tr>
<td>SysCheck Calibration Standard</td>
<td>301078</td>
</tr>
<tr>
<td>Bleach, minimum 5% or 0.7 M sodium hypochlorite solution</td>
<td>—</td>
</tr>
<tr>
<td>Disposable gloves</td>
<td>—</td>
</tr>
<tr>
<td>APTIMA Penetrable Caps</td>
<td>105668</td>
</tr>
<tr>
<td>Replacement non-penetrable caps</td>
<td>103036A</td>
</tr>
</tbody>
</table>
Optional Materials

<table>
<thead>
<tr>
<th>Item</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TECAN Freedom EVO 100/4 Instrument</td>
<td>900932</td>
</tr>
<tr>
<td>APTIMA Deck Plate Assembly, DTS 800</td>
<td>105200</td>
</tr>
<tr>
<td>Tips, 1000 µl conductive, liquid sensing</td>
<td>10612513 (Tecan)</td>
</tr>
<tr>
<td>Reagent reservoir (40 mL quarter module)</td>
<td>104765</td>
</tr>
<tr>
<td>Split reagent reservoir (19 mL x 2 quarter module)</td>
<td>901172</td>
</tr>
<tr>
<td>Bleach Enhancer for Cleaning</td>
<td>302101</td>
</tr>
</tbody>
</table>

DTS Systems Test Procedure

A. Work Area/Equipment Preparation

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

2. Place a sufficient number of Ten Tip Cassettes into the Target Capture System (TCS). Ensure that the TCS wash bottle is filled with Wash Solution and the aspiration manifold is connected to the vacuum pump. Refer to the Target Capture System Operator’s Manual.

3. Prepare the TECAN Freedom EVO instrument according to the instructions in its Operator’s Manual and HPV Application Sheet.

4. Prepare the pre-amplification SB100 instrument according to the instructions in its Operator’s Manual and HPV Application Sheet. Turn on the instrument and start the "APTIMA HPV PREAMP" protocol to allow the instrument to warm to 62°C.

5. Upon completion of the amplification step, prepare the post-amplification SB100 instrument according to the instructions in its Operator’s Manual and HPV Application Sheet. Turn on the instrument and start the "APTIMA HPV PSTAMP" protocol to allow the instrument to warm to 62°C.

6. Upon completion of the amplification step, prepare the LEADER HC+ Luminometer according the instructions in its Operator’s Manual after addition of the Probe Reagent as described in the Post Amplification steps.

B. Reagent Reconstitution/Preparation of a New Kit

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

1. To reconstitute the APTIMA HPV Enzyme, Amplification, and Probe Reagents, combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use:
   a. Pair each reconstitution solution with its lyophilized reagent. Ensure that the reconstitution solution and lyophilized reagent have matching label colors before attaching the reconstitution collar.
   b. Check the lot numbers on the Master Lot 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 bottle and set the cap on a clean, covered work surface.
e. While holding the solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 1, Step 2).

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

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

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

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

j. Recap the plastic bottle. Peel and discard the top label. Record required information on the remaining bottle label (Figure 1, Step 7).

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

Figure 1. DTS Systems Reconstitution Process

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

3. Prepare the Selection Reagent
   a. Check the reagent lot number on the Master Lot Sheet to make sure it belongs to the kit.
b. The Selection Reagent may precipitate if the temperature of the laboratory falls below 15°C or if the Selection Reagent has inadvertently been stored at 2°C to 8°C. Warm the Selection Reagent at 60°C for up to 45 minutes to facilitate dissolution of precipitate. Gently mix the bottle every 5 to 10 minutes. Allow the Selection Reagent to equilibrate to room temperature prior to use. Do not use if precipitate or cloudiness persists.

C. Reagent Preparation for Previously Reconstituted Reagents

1. Previously reconstituted Probe, Amplification, and Enzyme Reagents, must reach room temperature (15°C to 30°C) prior to the start of the assay.

2. If Probe Reagent contains precipitate that does not return to solution at room temperature, heat at 60°C for 1 to 2 minutes. After re-suspension, mix the vial by gentle inversion. Do not use if precipitate or cloudiness is present.

3. If wTCR contains precipitate, warm wTCR at 42°C to 60°C for up to 90 minutes. Allow the wTCR to equilibrate to room temperature prior to use.

4. If Selection Reagent contains precipitate, warm the Selection Reagent at 60°C 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 for cloudiness persists.

5. Thoroughly mix each reagent by gently inverting prior to use. Avoid creating foam during inversion of reagents.

D. Rack Setup

1. Allow the samples (calibrators, controls, and specimens) to reach room temperature prior to processing.

2. Do not vortex samples.

3. Inspect sample tubes before piercing them:
   a. If a sample tube contains bubbles in the space between the liquid and the cap, centrifuge the tube for 5 minutes at 420 RCF to eliminate the bubbles.
   b. If a specimen tube 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 steps 3a – 3b may result in liquid discharge from the specimen tube cap.

4. Place enough TTUs in the TTU rack to accommodate the calibrators, controls, and specimens.

5. (Optional) Create a worklist using the APTIMA Worklist Editor Software. Refer to the Worklist Editor section of the *APTIMA Assay Software Operator’s Manual* for specific instructions.

**Manual Pipetting Option**

1. Thoroughly mix the wTCR (TCR plus IC). Using a repeat pipettor, add 100 µL of wTCR to each reaction tube.

2. Using a micropipettor, pierce the cap of the sample tube, taking care not to drive the tip into the bottom of the tube.

3. Use a new pipette tip for each calibrator, control, and specimen.

4. Add 400 µL of the Negative Calibrator to the first three tubes in the first TTU.

5. Add 400 µL of the Positive Calibrator to tubes 4-6 of the first TTU.

6. Add 400 µL of the Negative Control to tube 7 of the first TTU.

7. Add 400 µL of the Positive Control to tube 8 of the first TTU.
8. Add 400 µL of each specimen to the remaining tubes.
9. When all samples have been pipetted, cover TTUs with sealing cards and proceed to Target Capture.

**TECAN Freedom EVO Instrument Option**

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

**E. Target Capture**

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

For information about the use of the GEN-PROBE Target Capture System, refer to the *Target Capture System Operator’s Manual*.

**Note:** The repeat pipettor used in target capture and amplification should be dedicated for use in these steps only. See Warnings and Precautions for more information.

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

10. Proceed to the Amplification step.

F. Amplification

1. Add Amplification reagent and Oil.

   Manual Pipetting Option
   a. Using the repeat pipettor, add 75 µL of the reconstituted Amplification Reagent to each reaction tube. All reaction mixtures in the rack should be red.
   b. Using the repeat pipettor, add 200 µL of Oil Reagent.
   c. Cover the tubes with sealing cards.
   d. Proceed to Step 2.

   TECAN Freedom EVO Instrument Option
   Refer to the TECAN Freedom EVO 100/4 Application Sheet for the APTIMA HPV Assay for specific instructions for the addition of Amplification and Oil reagents if using this instrument.

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

G. Post-Amplification

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

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

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

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

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

6. When the 5 minutes is up, as indicated by the SB100 display, add 250 µL Selection Reagent to each reaction tube using the repeat pipettor. All reaction mixtures should be pink.

7. Cover the tubes with sealing cards and the SB100 frame and ease the rack into the heating block. Press the appropriate key to start the vortex/incubation steps.

8. When the incubation is complete, remove the rack from the SB100 instrument and proceed to Detection.

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

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

7. Prepare Deactivation Fluid by mixing equal volumes of 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution and APTIMA Buffer for Deactivation Fluid in a large-capped plastic container. Label and write the expiration date on the plastic container. Deactivation Fluid is stable for 4 weeks at room temperature.

8. After removing the used TTUs from the luminometer, place the TTUs into the container with Deactivation Fluid. Allow the TTUs to sit in the container for 15 minutes before disposal. Proper handling and disposal methods should be established by the laboratory director.

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

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

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

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

D. Temperature

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

E. Time

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

F. Gloves

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

G. Decontamination

1. Laboratory bench surfaces and pipettors must be decontaminated regularly with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Allow the solution to contact surfaces for at least 1 minute, and then follow with a water rinse. Do not allow the solution to dry. Chlorine solutions may pit equipment and metal. Thoroughly rinse equipment with water to avoid pitting.

2. Decontaminate the TECAN Freedom EVO instrument according to the instructions in the Operators Manual.

3. Decontaminate the SB100 instruments per the instructions in the *SB100 Dry Heat Bath/Vortexer Application Sheet for the APTIMA HPV Assay*.

4. Decontaminate the Target Capture System per the instructions in the *Target Capture System Operator’s Manual*.

5. Wipe the surfaces of the TCS unit and wash buffer ejector tips with paper towels moistened with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Follow with a water rinse, and then dry the surfaces completely with paper towels.

6. Submerge the TTU racks in 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution, ensuring that they are covered by the solution. Keep the racks submerged for 10 minutes. Longer exposure could damage the racks. Rinse the racks thoroughly with water, place on a clean, absorbent pad, and allow to air-dry thoroughly. To prolong the life of the racks, allow to dry upright, not upside down.

7. TTUs must be decontaminated with Deactivation Fluid as described in the step for Detection. Do not reuse the TTUs.
Reagents for the APTIMA HPV Assay are listed below for the TIGRIS DTS System. Reagent Identification Symbols are also listed next to the reagent name.

Reagents and Materials Provided

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

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

**APTIMA HPV Refrigerated Box**
(store at 2°C to 8°C upon receipt)

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>HPV Amplification Reagent</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>Non-infectious nucleic acids dried in buffered solution containing &lt; 5% bulking agent.</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>HPV Enzyme Reagent</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing &lt; 10% bulking reagent.</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>HPV Probe Reagent</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>Non-infectious chemiluminescent DNA probes (&lt; 500 ng/vial) dried in succinate buffered solution containing &lt; 5% detergent.</td>
<td></td>
</tr>
<tr>
<td>IC</td>
<td>HPV Internal Control Reagent</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>RNA Transcript in buffered solution containing &lt; 5% detergent.</td>
<td></td>
</tr>
</tbody>
</table>

**APTIMA HPV Room Temperature Box**
(store at 15°C to 30°C upon receipt)

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR</td>
<td>HPV Amplification Reconstitution Solution</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>Aqueous solution containing preservatives.</td>
<td></td>
</tr>
<tr>
<td>ER</td>
<td>HPV Enzyme Reconstitution Solution</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>HEPES buffered solution containing a surfactant and glycerol.</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>HPV Probe Reconstitution Solution</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>Succinate buffered solution containing &lt; 5% detergent.</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>HPV Selection Reagent</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>600 mM borate buffered solution containing surfactant.</td>
<td></td>
</tr>
<tr>
<td>TCR</td>
<td>HPV Target Capture Reagent</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>Non-infectious nucleic acid in a buffered solution containing solid phase (&lt; 0.5 mg/mL).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reconstitution Collars</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Master Lot Barcode Sheet</td>
<td>1 sheet</td>
</tr>
</tbody>
</table>
Materials Required But Available Separately

Note: Materials available from Gen-Probe have catalog numbers listed, unless otherwise specified.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>HPV Positive Calibrator&lt;br&gt;Non-infectious HPV nucleic acid in a buffered solution containing &lt; 5% detergent.</td>
<td>5 vials</td>
</tr>
<tr>
<td>NC</td>
<td>HPV Negative Calibrator&lt;br&gt;Buffered solution containing &lt; 5% detergent.</td>
<td>5 vials</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>HPV Positive Control&lt;br&gt;Lysed, inactivated HPV Positive cultured cells in a buffered solution containing &lt; 5% detergent.</td>
<td>5 vials</td>
</tr>
<tr>
<td>NC</td>
<td>HPV Negative Control&lt;br&gt;Lysed, inactivated HPV Negative cultured cells in a buffered solution containing &lt; 5% detergent.</td>
<td>5 vials</td>
</tr>
</tbody>
</table>
APTIMA Penetrable Caps 105668
Replacement non-penetrable caps 103036
Spare Caps for Amplification and Probe Reagent reconstitution solutions CL0041
Spare Caps for Enzyme Reagent reconstitution solution 501616
Spare Caps for TCR and Selection Reagent CL0040

Optional Materials

Bleach Enhancer for Cleaning 302101

TIGRIS DTS System Test Procedure

Note: See the TIGRIS DTS System Operator’s Manual for additional TIGRIS DTS System procedural information.

A. Work Area Preparation

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

B. Reagent Preparation of a New Kit

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

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

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

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

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. The Selection Reagent may precipitate if the temperature of the laboratory falls below 15°C or if the Selection Reagent has inadvertently been stored at 2°C to 8°C. Warm the Selection Reagent at 60°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 Probe, Amplification, and Enzyme 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 for up to
45 minutes to facilitate dissolution of precipitate. Gently mix the bottle every 5 to 10
minutes. Allow the Selection Reagent to equilibrate to room temperature prior to use. Do
not use if precipitate or cloudiness persists.
   5. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid
creating foam during inversion of reagents.
   6. Do not top off reagent bottles. The TIGRIS DTS System will recognize and reject bottles
that have been topped off.

D. Sample Handling
   1. Allow the samples (calibrators, controls, and specimens) to reach room temperature
prior to processing.
   2. Do not vortex samples.
   3. Inspect sample tubes before loading into the rack:
      a. If a sample tube contains bubbles in the space between the liquid and the cap,
centrifuge the tube for 5 minutes at 420 RCF to eliminate the bubbles.
      b. If a specimen tube 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 steps 3a – 3b may result in liquid discharge from the specimen
tube cap.

E. System Preparation
   Set up the instrument and worklist according to the instructions in the TIGRIS DTS System
Operator’s Manual and the Procedural Notes section below.

Procedural Notes

A. Calibrators
   1. Each worklist must contain 3 replicates of the Negative Calibrator and Positive
Calibrator. The APTIMA HPV Assay Software is designed so that the analyzer pipets
the replicates from each calibrator tube three times. In order to work properly with the
APTIMA HPV Assay Software, the Negative Calibrator must be in the first tube position
of the first rack of the worklist and the Positive Calibrator must be in the second tube
position of the first rack of the worklist.
   2. Attempts to pipette more than three replicates from a calibrator tube can lead to
insufficient volume errors.

B. Controls
   1. The APTIMA HPV Assay software requires beginning and end of run controls. The
Negative Control must be in the third tube position of the first rack and the second to last
tube position of the last rack of the worklist. The Positive Control must be in the fourth tube position of the first rack and the last tube position of the last rack of the worklist.

2. Attempts to pipette more than once from a control tube can lead to insufficient volume errors.

C. Temperature

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

D. Gloves

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

E. Rack Decontamination

Submerge the racks in 2.5% to 3.5% sodium hypochlorite solution (diluted bleach), ensuring that they are covered by the bleach solution. Keep the racks submerged for 10 minutes. Longer exposure will damage the racks. Rinse the racks thoroughly with water and place on a clean absorbent pad; allow the racks to air-dry thoroughly.
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)

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>HPV Amplification Reagent</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>Non-infectious nucleic acids dried in buffered solution containing &lt; 5% bulking agent.</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>HPV Enzyme Reagent</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing &lt; 10% bulking reagent.</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>HPV Probe Reagent</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>Non-infectious chemiluminescent DNA probes (&lt; 500 ng/vial) dried in succinate buffered solution containing &lt; 5% detergent.</td>
<td></td>
</tr>
<tr>
<td>IC</td>
<td>HPV Internal Control Reagent</td>
<td>1 vial</td>
</tr>
<tr>
<td></td>
<td>RNA Transcript in buffered solution containing &lt; 5% detergent.</td>
<td></td>
</tr>
</tbody>
</table>

### APTIMA HPV Room Temperature Box

(Store at room temperature, 15°C to 30°C upon receipt)

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR</td>
<td>HPV Amplification Reconstitution Solution</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Aqueous solution containing preservatives.</td>
<td></td>
</tr>
<tr>
<td>ER</td>
<td>HPV Enzyme Reconstitution Solution</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>HEPES buffered solution containing a surfactant and glycerol.</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>HPV Probe Reconstitution Solution</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Succinate buffered solution containing &lt; 5% detergent.</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>HPV Selection Reagent</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>600 mM borate buffered solution containing surfactant.</td>
<td></td>
</tr>
<tr>
<td>TCR</td>
<td>HPV Target Capture Reagent</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Non-infectious nucleic acid in a buffered solution containing solid phase (&lt; 0.5 mg/mL).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reconstitution Collars</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Master Lot Barcode Sheet</td>
<td>1 sheet</td>
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</table>
Materials Required But Available Separately

Note: Materials available from Gen-Probe have catalog numbers listed, unless otherwise specified.

### APTIMA HPV Calibrators Box (Cat. No. 303011)
(store at 2°C to 8°C upon receipt)

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Component</th>
<th>Quantity</th>
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</thead>
<tbody>
<tr>
<td>PCAL</td>
<td>HPV Positive Calibrator  &lt;br&gt;Non-infectious HPV nucleic acid in a buffered solution containing &lt; 5% detergent.</td>
<td>5 vials</td>
</tr>
<tr>
<td>NCAL</td>
<td>HPV Negative Calibrator &lt;br&gt;Buffered solution containing &lt; 5% detergent.</td>
<td>5 vials</td>
</tr>
</tbody>
</table>

### Optional Materials

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Bleach Enhancer for Cleaning</th>
</tr>
</thead>
</table>
PANTHER System Test Procedure

Note: See the PANTHER System Operator’s Manual for additional PANTHER System procedural information.

A. Work Area Preparation

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

B. Reagent Preparation of a New Kit

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

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

**Figure 3. PANTHER System Reconstitution Process**

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

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. The Selection Reagent may precipitate if the temperature of the laboratory falls below 15°C or if the Selection Reagent has inadvertently been stored at 2°C to 8°C. Warm the Selection Reagent at 60°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 Probe, Amplification, and Enzyme 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 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 thoroughly by gently inverting prior to loading onto the system. Avoid creating foam during inversion of reagents.

6. Do not top off reagent bottles. The PANTHER System will recognize and reject bottles that have been topped off.

D. Sample Handling

1. Allow the samples (calibrators and specimens) to reach room temperature prior to processing.

2. Do not vortex specimens.

3. Inspect sample tubes before loading into the rack:
   a. If a sample tube contains bubbles in the space between the liquid and the cap, centrifuge the tube for 5 minutes at 420 RCF to eliminate the bubbles.
   b. If a specimen tube 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 steps 3a – 3b may result in liquid discharge from the specimen tube cap.

E. System Preparation

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

2. Load samples.

Procedural Notes

A. Calibrators

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

2. Once the calibrator tubes have been pipetted and are being processed for a specific reagent kit, specimens can be run with the associated assay reagent kit for up to 24 hours unless:
   a. Calibrators are invalid.
   b. The associated assay reagent kit is removed from the system.
c. The associated assay reagent kit has exceeded the stability limits.

3. Attempts to pipette more than three replicates from a calibrator tube can lead to processing errors.

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

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

A. Run Validity Criteria

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

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

A run may be invalidated by an operator if technical, operator, or instrument difficulties are observed and documented while performing the assay.

Note: An invalid run must be repeated.

B. Calibrator Acceptance Criteria

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

<table>
<thead>
<tr>
<th></th>
<th>Negative Calibrator</th>
<th>Positive Calibrator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyte RLU</td>
<td>≥ 0 and ≤ 45,000 RLU</td>
<td>≥ 480,000 and ≤ 1,850,000 RLU</td>
</tr>
<tr>
<td>Internal Control</td>
<td>≥ 75,000 and ≤ 400,000 RLU</td>
<td>≤ 450,000 RLU</td>
</tr>
</tbody>
</table>

C. Internal Control Cutoff Calculation

The Internal Control cutoff is determined from the Internal Control (flasher) signal from 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.

E. Control Acceptance Criteria (DTS Systems and TIGRIS DTS System only)

The Negative Control must have a valid (IC RLU ≥ IC cutoff) negative (analyte S/CO < 0.50) result. The Positive Control must have a valid positive (analyte S/CO ≥ 0.50) result.
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 signal-to-cutoff (S/CO) for the Internal Control and 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 Cervical Specimen Collection and Transport (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:** If using the TECAN EVO instrument for sample pipetting with the DTS Systems, or testing on the TIGRIS DTS System or the PANTHER System, 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.

<table>
<thead>
<tr>
<th>Specimen Interpretation</th>
<th>Criteria</th>
</tr>
</thead>
</table>
| **Negative**            | Analyte S/CO < 0.50  
Internal Control ≥ IC Cutoff  
Internal Control ≤ 2,000,000 RLU |
| **Positive**            | Analyte S/CO ≥ 0.50  
Internal Control ≤ 2,000,000 RLU  
Analyte ≤ 13,000,000 RLU |
| **Invalid**             | Internal Control > 2,000,000 RLU  
or  
Analyte S/CO < 0.50 and Internal Control < IC Cutoff  
or  
Analyte > 13,000,000 RLU |
Limitations

A. The APTIMA HPV Assay has been validated on the DTS Systems and the TIGRIS DTS System using only clinician-collected APTIMA Cervical Specimen Collection and Transport Kit and PreservCyt liquid Pap specimens. Performance with other specimen types has not been evaluated.

B. The APTIMA HPV Assay has been validated on the PANTHER System using only the clinician-collected APTIMA Cervical Specimen Collection and Transport Kit.

C. Test results may be affected by improper specimen collection or specimen processing.

D. Results from the APTIMA HPV Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

E. The APTIMA HPV Assay has not been evaluated in cases of suspected sexual abuse.

F. Prevalence of HPV infection in a population affects interpretation of a test’s performance. Positive predictive values decrease when testing populations with low prevalence or individuals with no risk of infection.

G. PreservCyt liquid Pap specimens containing less than 1 mL after ThinPrep Pap Test slide preparation are considered inadequate for the APTIMA HPV Assay.

H. APTIMA HPV Assay performance has not been evaluated with post-processed PreservCyt specimens using processors other than the ThinPrep 2000 Processor.

I. A negative APTIMA HPV Assay result does not exclude the possibility 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% 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% 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.
**DTS Systems Assay Performance**

**Clinical Performance**

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

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

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

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTIMA HPV</td>
<td>412</td>
<td>5</td>
<td>417</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>331</td>
<td>364</td>
</tr>
<tr>
<td>Total</td>
<td>445</td>
<td>336</td>
<td>781</td>
</tr>
</tbody>
</table>

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

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

Similar analyses were also performed using a clinical endpoint of CIN3+. The APTIMA HPV Assay clinical sensitivity and specificity for detection of CIN3+ is shown in Table 3A for the 753 specimens with histology results tested on the DTS Systems. The clinical sensitivity of the assay was 97.7%, the specificity was 52.9%, and the positive and negative predictive values for detection of CIN3+ were 21.3% and 99.4%, respectively. Again, APTIMA HPV Assay sensitivity was similar to that of HPV-DNA, for which the sensitivity for detection of CIN3+ was 98.9% (Table 3B) and APTIMA HPV Assay specificity was significantly higher than HPV-DNA specificity, which was 44.4% in this population for detection of CIN3+ lesions. Of the 753 specimens with histology results, 159 had an ASCUS cytology result. The sensitivity and specificity of the APTIMA HPV Assay in this population was 100% and 40.1% respectively for detection of CIN3+.
These results, which yielded similar sensitivity and significantly higher specificity for the APTIMA HPV Assay, as compared to high-risk DNA detection, are similar to results obtained in other studies.\textsuperscript{14,15,16,17,18}

**Table 2A: Sensitivity and Specificity of the APTIMA HPV Assay on the DTS Systems for Detection of Disease (CIN2+)**

<table>
<thead>
<tr>
<th></th>
<th>CIN2+</th>
<th>&lt; CIN2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>APTIMA HPV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>128</td>
<td>271</td>
<td>399</td>
</tr>
<tr>
<td>-</td>
<td>13</td>
<td>341</td>
<td>354</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>141</td>
<td>612</td>
<td>753</td>
</tr>
</tbody>
</table>

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

**Table 2B: Sensitivity and Specificity of the HPV-DNA assay for Detection of Disease (CIN2+)**

<table>
<thead>
<tr>
<th></th>
<th>CIN2+</th>
<th>&lt; CIN2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HPV-DNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>134</td>
<td>322</td>
<td>456</td>
</tr>
<tr>
<td>-</td>
<td>7</td>
<td>290</td>
<td>297</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>141</td>
<td>612</td>
<td>753</td>
</tr>
</tbody>
</table>

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

**Table 3A: Sensitivity and Specificity of the APTIMA HPV Assay on the DTS Systems for Detection of Disease (CIN3+)**

<table>
<thead>
<tr>
<th></th>
<th>CIN3+</th>
<th>&lt; CIN3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>APTIMA HPV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>85</td>
<td>314</td>
<td>399</td>
</tr>
<tr>
<td>-</td>
<td>2</td>
<td>352</td>
<td>354</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>87</td>
<td>666</td>
<td>753</td>
</tr>
</tbody>
</table>

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

**Table 3B: Sensitivity and Specificity of the HPV-DNA assay for Detection of Disease (CIN3+)**

<table>
<thead>
<tr>
<th></th>
<th>CIN3+</th>
<th>&lt; CIN3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HPV-DNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>86</td>
<td>370</td>
<td>456</td>
</tr>
<tr>
<td>-</td>
<td>1</td>
<td>296</td>
<td>297</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>87</td>
<td>666</td>
<td>753</td>
</tr>
</tbody>
</table>

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

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

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

Table 4: Overall Agreement of APTIMA HPV Assay results from PreservCyt liquid Pap specimens and APTIMA Cervical Specimen Collection and Transport Kit specimens tested on the DTS Systems

<table>
<thead>
<tr>
<th>PreservCyt liquid Pap specimen</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTIMA Cervical Specimen Collection and Transport Kit specimen</td>
<td>+</td>
<td>233</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>12</td>
<td>463</td>
</tr>
<tr>
<td>Total</td>
<td>245</td>
<td>483</td>
<td>728</td>
</tr>
</tbody>
</table>

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

Analytical Sensitivity

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

Probit regression analysis shows that HPV 16, 18, 31, 33, 35, 39, 45, 56, 58, 59, 66 and 68 had predicted 95% detection limits less than 100 copies/reaction; and types 51 and 52 had predicted 95% detection limits between 100 and 300 copies/reaction.
**Table 5: Predicted 95% Detection Limit of the APTIMA HPV Assay determined by Probit Analysis of the DTS Systems Data**

<table>
<thead>
<tr>
<th>Target</th>
<th>95% Detection Limit* (95% Fiducial Limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV 16</td>
<td>74 (54 - 113)</td>
</tr>
<tr>
<td>HPV 18</td>
<td>52 (39 - 76)</td>
</tr>
<tr>
<td>HPV 31</td>
<td>19 (14 - 27)</td>
</tr>
<tr>
<td>HPV 33</td>
<td>24 (18 - 37)</td>
</tr>
<tr>
<td>HPV 35</td>
<td>27 (22 - 38)</td>
</tr>
<tr>
<td>HPV 39</td>
<td>32 (23 - 49)</td>
</tr>
<tr>
<td>HPV 45</td>
<td>28 (17 - 90)</td>
</tr>
<tr>
<td>HPV 51</td>
<td>198 (147 - 289)</td>
</tr>
<tr>
<td>HPV 52</td>
<td>239 (187 - 324)</td>
</tr>
<tr>
<td>HPV 56</td>
<td>48 (36 - 71)</td>
</tr>
<tr>
<td>HPV 58</td>
<td>99 (74 - 146)</td>
</tr>
<tr>
<td>HPV 59</td>
<td>89 (68 - 127)</td>
</tr>
<tr>
<td>HPV 68</td>
<td>27 (20 - 40)</td>
</tr>
<tr>
<td>HPV 66</td>
<td>68 (50 - 105)</td>
</tr>
<tr>
<td>SiHa cells</td>
<td>0.58 (0.44 - 0.87)</td>
</tr>
<tr>
<td>HeLa cells</td>
<td>0.04 (0.03 - 0.06)</td>
</tr>
<tr>
<td>ME180 cells</td>
<td>0.02 (0.01 - 0.03)</td>
</tr>
<tr>
<td>MS751 cells</td>
<td>0.03 (0.02 - 0.04)</td>
</tr>
</tbody>
</table>

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

**Assay Reproducibility**

The reproducibility of the APTIMA HPV Assay was determined by testing 16 panel members in triplicate in 2 runs with 2 reagent lots, on 3 instruments by 3 operators. Testing was conducted over 20 days at one site. The panel members are described in Table 6. Six of the panel members were HPV negative (3 were GEN-PROBE specimen transport media and 3 were pooled PreservCyt liquid Pap specimens), four were HPV low positive (~95% detection limit), and six were HPV moderate positive (≥ ~3x the 95% detection limit). The low positive and moderate positive panel members were comprised of either in vitro transcript (IVT) or HPV infected cultured cells in GEN-PROBE specimen transport media.

**Table 6: APTIMA HPV Assay Reproducibility Panel**

<table>
<thead>
<tr>
<th>Panel Member</th>
<th>Description</th>
<th>Concentration</th>
<th>Expected HPV Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>STM Lot 1</td>
<td>N/A</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>SiHa Low Positive</td>
<td>1 cell/rxn</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>HeLa Low Positive</td>
<td>0.15 cell/rxn</td>
<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>Clinical Pool 1</td>
<td>N/A</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>ME180 Moderate Positive</td>
<td>1 cell/rxn</td>
<td>Positive</td>
</tr>
<tr>
<td>6</td>
<td>MS751 Moderate Positive</td>
<td>1 cell/rxn</td>
<td>Positive</td>
</tr>
</tbody>
</table>
One hundred and eight data points for each reproducibility panel member was analyzed for the DTS Systems, the results for which are summarized in Table 7. The percent positive for the negative panels ranged from 0 to 3.7; low positive was ≥ 98; and the moderate positive was 100. The agreement with the expected result was > 96% for all of the panel members.

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

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

Table 7: APTIMA HPV Assay Reproducibility on the DTS Systems

<table>
<thead>
<tr>
<th>Panel Member</th>
<th>Description</th>
<th>Concentration</th>
<th>Expected HPV Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>SiHa &amp; HeLa Moderate Positive</td>
<td>10 cell/rxn &amp; 1 cell/rxn</td>
<td>Positive</td>
</tr>
<tr>
<td>8</td>
<td>STM Lot 2</td>
<td>N/A</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>Clinical Pool 2</td>
<td>N/A</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>HPV 16 IVT Low Positive</td>
<td>30 copies/rxn</td>
<td>Positive</td>
</tr>
<tr>
<td>11</td>
<td>HPV 18 IVT Low Positive</td>
<td>30 copies/rxn</td>
<td>Positive</td>
</tr>
<tr>
<td>12</td>
<td>STM Lot 3</td>
<td>N/A</td>
<td>Negative</td>
</tr>
<tr>
<td>13</td>
<td>HPV 16 IVT Moderate Positive</td>
<td>100 copies/rxn</td>
<td>Positive</td>
</tr>
<tr>
<td>14</td>
<td>HPV 18 IVT Moderate Positive</td>
<td>100 copies/rxn</td>
<td>Positive</td>
</tr>
<tr>
<td>15</td>
<td>HPV 16 &amp; HPV 18 Moderate Positive</td>
<td>100/100 copies/rxn</td>
<td>Positive</td>
</tr>
<tr>
<td>16</td>
<td>Clinical Pool 3</td>
<td>N/A</td>
<td>Negative</td>
</tr>
</tbody>
</table>
Table 7: APTIMA HPV Assay Reproducibility on the DTS Systems (continued)

<table>
<thead>
<tr>
<th>Panel Member</th>
<th>Description</th>
<th>N</th>
<th>% Positive</th>
<th>Agreement</th>
<th>Mean S/CO</th>
<th>Inter-Instrument SD</th>
<th>Inter-Operator SD</th>
<th>Inter-Lot SD</th>
<th>Inter-Run SD</th>
<th>Intra-Run SD</th>
<th>Total SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Low pos</td>
<td>108</td>
<td>99.1%</td>
<td>99.1%</td>
<td>N/A</td>
<td>10.61</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>11</td>
<td>Low pos</td>
<td>108</td>
<td>98.1%</td>
<td>98.1%</td>
<td>N/A</td>
<td>9.04</td>
<td>0.0</td>
<td>0.0</td>
<td>0.4</td>
<td>4.1</td>
<td>0.0</td>
</tr>
<tr>
<td>12</td>
<td>Neg</td>
<td>108</td>
<td>0.0%</td>
<td>100%</td>
<td>1.85</td>
<td>0.00</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>13</td>
<td>Mod pos</td>
<td>108</td>
<td>100%</td>
<td>100%</td>
<td>N/A</td>
<td>10.99</td>
<td>0.1</td>
<td>1.4</td>
<td>0.1</td>
<td>0.8</td>
<td>0.0</td>
</tr>
<tr>
<td>14</td>
<td>Mod pos</td>
<td>108</td>
<td>100%</td>
<td>100%</td>
<td>N/A</td>
<td>12.22</td>
<td>0.3</td>
<td>2.6</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>15</td>
<td>Mod pos</td>
<td>108</td>
<td>100%</td>
<td>100%</td>
<td>N/A</td>
<td>23.37</td>
<td>0.7</td>
<td>2.8</td>
<td>0.3</td>
<td>1.5</td>
<td>0.0</td>
</tr>
<tr>
<td>16</td>
<td>Neg</td>
<td>108</td>
<td>0.9%</td>
<td>99.1%</td>
<td>1.79</td>
<td>0.03</td>
<td>0.0</td>
<td>2.3</td>
<td>0.0</td>
<td>1.7</td>
<td>0.0</td>
</tr>
</tbody>
</table>

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

Cross-Reactivity

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

Table 8: Analytical Specificity Panel

<table>
<thead>
<tr>
<th>Organism</th>
<th>Test Concentration</th>
<th>Organism</th>
<th>Test Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter lwofii</td>
<td>1x10^8 CFU/mL</td>
<td>Klebsiella pneumoniae</td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td>Actinomyces israelii</td>
<td>1x10^8 CFU/mL</td>
<td>Lactobacillus acidophilus</td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td>Adenovirus 2</td>
<td>1x10^4 vp/mL</td>
<td>Lactobacillus crispatus</td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td>Alcaligenes faecalis</td>
<td>1x10^4 CFU/mL</td>
<td>Lactobacillus delbrueckii ssp. bulgaricus</td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td>Atopobium vaginae</td>
<td>5x10^7 CFU/mL</td>
<td>Lactobacillus jensenii</td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>1x10^6 CFU/mL</td>
<td>Listeria monocytogenes</td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td>Bacteroides fragilis</td>
<td>1x10^6 CFU/mL</td>
<td>Micrococcus luteus</td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td>Bacteroides ureolyticus</td>
<td>1x10^6 CFU/mL</td>
<td>Mobiluncus curtisi</td>
<td>2x10^7 CFU/mL</td>
</tr>
<tr>
<td>Bifidobacterium adolescentis</td>
<td>1x10^6 CFU/mL</td>
<td>Mycobacterium smegmatis</td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td>Bifidobacterium breve</td>
<td>1x10^6 CFU/mL</td>
<td>Mycoplasma fermentans</td>
<td>5x10^7 CFU/mL</td>
</tr>
<tr>
<td>Campylobacter fetus-fetus</td>
<td>1x10^6 CFU/mL</td>
<td>Mycoplasma genitalium</td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>1x10^6 CFU/mL</td>
<td>Mycoplasma hominis</td>
<td>5x10^7 CFU/mL</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>2x10^4 TCID 50/mL</td>
<td>Neisseria gonorrhoeae</td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>6x10^7 CFU/mL</td>
<td>Neisseria gonorrhoeae and Chlamydia trachomatis</td>
<td>5x10^7 CFU/mL 1.5x10^4 TCID 50/mL</td>
</tr>
</tbody>
</table>
### Table 8: Analytical Specificity Panel (continued)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Test Concentration</th>
<th>Organism</th>
<th>Test Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>1x10⁸ CFU/mL</td>
<td><em>Neisseria meningitidis</em></td>
<td>1x10⁸ CFU/mL</td>
</tr>
<tr>
<td><em>Corynebacterium genitalium</em></td>
<td>1x10⁸ CFU/mL</td>
<td><em>Peptostreptococcus anaerobius</em></td>
<td>1x10⁸ CFU/mL</td>
</tr>
<tr>
<td><em>Corynebacterium xerosis</em></td>
<td>1x10⁸ CFU/mL</td>
<td><em>Propionibacterium acnes</em></td>
<td>1x10⁸ CFU/mL</td>
</tr>
<tr>
<td><em>Cytomegalovirus</em></td>
<td>33 TCID 50/mL</td>
<td><em>Proteus mirabilis</em></td>
<td>1x10⁸ CFU/mL</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>1x10⁸ CFU/mL</td>
<td><em>Proteus vulgaris</em></td>
<td>1x10⁸ CFU/mL</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>1x10⁸ CFU/mL</td>
<td><em>Providencia stuartii</em></td>
<td>1x10⁸ CFU/mL</td>
</tr>
<tr>
<td><em>Epstein-Barr virus</em></td>
<td>4x10⁷ vp/mL</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>1x10⁸ CFU/mL</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>1x10⁸ CFU/mL</td>
<td><em>Ruminococcus productus</em></td>
<td>1x10⁸ CFU/mL</td>
</tr>
<tr>
<td><em>Fusobacterium nucleatum</em></td>
<td>1x10⁸ CFU/mL</td>
<td><em>Serratia marcescens</em></td>
<td>1x10⁸ CFU/mL</td>
</tr>
<tr>
<td><em>Gardnerella vaginalis</em></td>
<td>1x10⁸ CFU/mL</td>
<td><em>Staphylococcus aureus</em></td>
<td>1x10⁸ CFU/mL</td>
</tr>
<tr>
<td><em>Haemophilus ducreyi</em></td>
<td>1x10⁸ CFU/mL</td>
<td><em>Staphylococcus epidermidis</em></td>
<td>1x10⁸ CFU/mL</td>
</tr>
<tr>
<td><em>Herpes simplex virus 1</em></td>
<td>2.5x10⁸ TCID 50/mL</td>
<td><em>Staphylococcus saprophyticus</em></td>
<td>1x10⁸ CFU/mL</td>
</tr>
<tr>
<td><em>Herpes simplex virus 2</em></td>
<td>5x10⁸ TCID 50/mL</td>
<td><em>Streptococcus agalactiae</em></td>
<td>1x10⁸ CFU/mL</td>
</tr>
<tr>
<td><em>HIV-1</em></td>
<td>1.0x10⁶ copies/mL</td>
<td><em>Streptococcus pyogenes</em></td>
<td>1x10⁸ CFU/mL</td>
</tr>
<tr>
<td><em>HPV 6</em></td>
<td>2.5x10⁶ copies/mL</td>
<td><em>Streptococcus sanguinis</em></td>
<td>1x10⁸ CFU/mL</td>
</tr>
<tr>
<td><em>HPV 11</em></td>
<td>2.5x10⁶ copies/mL</td>
<td><em>SV40</em></td>
<td>1.2 x 10⁷ TCID 50/mL</td>
</tr>
<tr>
<td><em>HPV 42</em></td>
<td>2.5x10⁶ copies/mL</td>
<td><em>Trichomonas vaginalis</em></td>
<td>1x10⁶ cells/mL</td>
</tr>
<tr>
<td><em>HPV 43</em></td>
<td>2.5x10⁶ copies/mL</td>
<td><em>Ureaplasma urealyticum</em></td>
<td>1x10⁸ CFU/mL</td>
</tr>
<tr>
<td><em>HPV 44</em></td>
<td>2.5x10⁶ copies/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>HPV 53</em></td>
<td>2.5x10⁶ copies/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>HPV 61</em></td>
<td>2.5x10⁶ copies/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>HPV 71</em></td>
<td>2.5x10⁶ copies/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>HPV 81</em></td>
<td>2.5x10⁶ copies/mL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Interference

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

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

*Personal lubricants that contain Polyquaternium 15.*
TIGRIS DTS System Assay Performance

Clinical Performance

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

The APTIMA HPV Assay sensitivity and specificity for detection of high-risk HPV is shown in Table 10 for the 780 specimens tested on the TIGRIS DTS system. The sensitivity of the assay was 93.5%, the specificity was 98.8%, and the positive and negative predictive values for detection of high-risk HPV were 99.0% and 91.9% respectively.

Table 10: Sensitivity and Specificity of the APTIMA HPV Assay on the TIGRIS DTS System for Detection of High-risk HPV

<table>
<thead>
<tr>
<th>High-Risk HPV</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTIMA HPV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>417</td>
<td>4</td>
</tr>
<tr>
<td>-</td>
<td>29</td>
<td>330</td>
</tr>
<tr>
<td>Total</td>
<td>446</td>
<td>334</td>
</tr>
</tbody>
</table>

Sensitivity (95% CI) = 93.5% (90.8-95.4)
Specificity (95% CI) = 98.8% (97.0-99.5)
Positive Predictive Value = 99.0%
Negative Predictive Value = 91.9%

The APTIMA HPV Assay clinical sensitivity and specificity for detection of CIN2+ is shown in Table 11 for the 753 specimens with histology results tested on the TIGRIS DTS System. The clinical sensitivity of the assay was 90.8%, the specificity was 54.7%, and the positive and negative predictive values for detection of CIN2+ were 31.6% and 96.3% respectively. These results were similar to those observed on the DTS Systems, for which APTIMA HPV Assay sensitivity was similar to HPV-DNA, with higher specificity than HPV-DNA. Of the 753 specimens with histology results, 159 had an ASCUS cytology result. The sensitivity and specificity of the APTIMA HPV Assay in this population was 96.2% and 39.1% respectively.

Similar analyses were also performed using a clinical endpoint of CIN3+. The APTIMA HPV Assay clinical sensitivity and specificity for detection of CIN3+ is shown in Table 12 for the 753 specimens with histology results tested on the TIGRIS DTS System. The clinical sensitivity of the assay was 97.7%, the specificity was 52.0%, and the positive and negative predictive values for detection of CIN3+ were 21.0% and 99.4% respectively. Again, these results were similar to those observed on the DTS Systems, with APTIMA HPV Assay sensitivity similar to HPV-DNA, with specificity significantly higher than HPV-DNA for detection of CIN3+ in this population. Of the 753 specimens with histology...
results, 159 had an ASCUS cytology result. The sensitivity and specificity of the APTIMA HPV Assay in this population was 100% and 37.3% respectively for detection of CIN3+.

Table 11: Sensitivity and Specificity of the APTIMA HPV Assay on the TIGRIS DTS System for Detection of Disease (CIN2+)

<table>
<thead>
<tr>
<th>TIGRIS DTS System Assay Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTIMA HPV</td>
</tr>
<tr>
<td>+ 128</td>
</tr>
<tr>
<td>- 13</td>
</tr>
<tr>
<td>Total 141</td>
</tr>
</tbody>
</table>

Sensitivity (95% CI) = 90.8% (84.9-94.5)
Specificity (95% CI) = 54.7% (50.8-58.6)
Positive Predictive Value = 31.6%
Negative Predictive Value = 96.3%

Table 12: Sensitivity and Specificity of the APTIMA HPV Assay on the TIGRIS DTS System for Detection of Disease (CIN3+)

<table>
<thead>
<tr>
<th>CIN3+</th>
<th>&lt; CIN3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTIMA HPV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ 85</td>
<td>320</td>
<td>405</td>
</tr>
<tr>
<td>- 2</td>
<td>346</td>
<td>348</td>
</tr>
<tr>
<td>Total 87</td>
<td>666</td>
<td>753</td>
</tr>
</tbody>
</table>

Sensitivity (95% CI) = 97.7% (92.0-99.4)
Specificity (95% CI) = 52.0% (48.2-55.7)
Positive Predictive Value = 21.0%
Negative Predictive Value = 99.4%

Specimen Comparison

Paired PreservCyt liquid Pap specimens and APTIMA Cervical Specimen Collection and Transport (CSCT) Kit specimens were collected from 735 subjects. One milliliter (1.0 mL) of each PreservCyt 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 PreservCyt liquid Pap specimen and the CSCT specimen was determined and the results are shown in Table 13.

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 Pap and transport kit specimens was observed (kappa = 0.90).

Table 13: Overall Agreement of APTIMA HPV Assay results from PreservCyt liquid Pap specimens and APTIMA Cervical Specimen Collection and Transport Kit specimens tested on the TIGRIS DTS System

<table>
<thead>
<tr>
<th>PreservCyt liquid Pap speciemen</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTIMA Cervical Specimen Collection and Transport Kit specimen</td>
<td>234</td>
<td>22</td>
<td>256</td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>469</td>
<td>479</td>
</tr>
<tr>
<td>Total</td>
<td>244</td>
<td>491</td>
<td>735</td>
</tr>
</tbody>
</table>

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

The analytical sensitivity of the APTIMA HPV Assay for detection of high-risk HPV was determined by testing individual negative clinical PreservCyt liquid Pap specimens spiked with HPV in vitro transcripts or infected cells at various concentrations. Sixty replicates of each copy level were tested. Probit regression analysis was performed and the predicted 95% detection limit determined for each HPV type (Table 14).

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

Table 14: Predicted 95% Detection Limit of the APTIMA HPV Assay Determined by Probit Analysis of TIGRIS DTS System Data

<table>
<thead>
<tr>
<th>Target</th>
<th>95% Detection Limit*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(95% Fiducial Limits)</td>
</tr>
<tr>
<td>HPV 16</td>
<td>34 (26-48)</td>
</tr>
<tr>
<td>HPV 18</td>
<td>55 (42 - 80)</td>
</tr>
<tr>
<td>HPV 31</td>
<td>18 (13 - 27)</td>
</tr>
<tr>
<td>HPV 33</td>
<td>27 (21 - 38)</td>
</tr>
<tr>
<td>HPV 35</td>
<td>41 (30 - 62)</td>
</tr>
<tr>
<td>HPV 39</td>
<td>12 (9 - 18)</td>
</tr>
<tr>
<td>HPV 45</td>
<td>42 (31 - 66)</td>
</tr>
<tr>
<td>HPV 51</td>
<td>96 (78 - 126)</td>
</tr>
<tr>
<td>HPV 52</td>
<td>168 (131 - 237)</td>
</tr>
<tr>
<td>HPV 56</td>
<td>37 (30 - 50)</td>
</tr>
<tr>
<td>HPV 58</td>
<td>56 (43 - 81)</td>
</tr>
<tr>
<td>HPV 59</td>
<td>60 (44 - 93)</td>
</tr>
<tr>
<td>HPV 66</td>
<td>81 (64 - 114)</td>
</tr>
<tr>
<td>HPV 68</td>
<td>30 (24 - 41)</td>
</tr>
<tr>
<td>SiHa cells</td>
<td>0.70 (0.52 - 1.04)</td>
</tr>
<tr>
<td>HeLa cells</td>
<td>0.04 (0.03 - 0.06)</td>
</tr>
<tr>
<td>ME180 cells</td>
<td>0.03 (0.03 - 0.05)</td>
</tr>
<tr>
<td>MS751 cells</td>
<td>0.04 (0.03 - 0.06)</td>
</tr>
</tbody>
</table>

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

Assay Reproducibility

The reproducibility of the APTIMA HPV Assay was determined by testing 16 panel members in triplicate in 2 runs with 2 reagent lots, on 3 instruments by 3 operators. Testing was conducted over 20 days at one site. The panel members are described in Table 15. Six of the panel members were HPV negative (3 were GEN-PROBE specimen transport media and 3 were pooled PreservCyt liquid Pap specimens), four were HPV low positive (~95% detection limit), and six were HPV moderate positive (≥ ~ 3x the 95% detection limit). The low positive and moderate positive panel members were
comprised of either in vitro transcript (IVT) or HPV infected cultured cells in GEN-PROBE specimen transport media.

**Table 15: APTIMA HPV Assay Reproducibility Panel**

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

One hundred and eight data points for each reproducibility panel member was analyzed for the TIGRIS DTS System, the results for which are summarized in Table 16. The percent agreement for the negative panels was > 97%. For low and moderate positives, the agreement was 100%.

The mean internal control S/CO was determined for the 6 negative panel members (1, 4, 8, 9, 12 and 16); the inter-instrument, -operator, -lot, and -run variability was calculated, as well as the intra-run variability. The mean IC S/CO for the negative panel members ranged from 1.93 to 2.08. The coefficient of variation (CV) for the IC S/CO values was quite low, < 10% for all parameters evaluated. The variability of the analyte S/CO values for the negative panels was not analyzed for the negative panel members due to the inherent variability when values of zero are observed.

The mean analyte S/CO was determined for the 10 positive panel members (2-3, 5-7, 10-11, and 13-15); the inter-instrument, -operator, -lot, and -run variability was calculated, as well as the intra-run variability. The mean analyte S/CO values ranged from 9.43 to 10.95 for the low positive panels and 9.07 to 15.44 for the moderate positive panels. The two panel members containing 2 high-risk HPV types, panel 7 and 15, had mean analyte S/CO values of 22.15 and 22.97 respectively. The CVs for the low positive and moderate positive panel members were < 30% and < 15% respectively, with the highest variability observed within a run. The internal control S/CO values were not evaluated for the positive panel members because the internal control RLU are not indicative of an individual reaction’s performance in an analyte positive sample.
### Table 16: APTIMA HPV Assay Analyte Reproducibility on the TIGRIS DTS System

<table>
<thead>
<tr>
<th>Panel Member Description</th>
<th>N</th>
<th>Positive Agreement</th>
<th>Mean S/CO</th>
<th>Inter-Instrument SD</th>
<th>Inter-Operator SD</th>
<th>Inter-Lot SD</th>
<th>Inter-Run SD</th>
<th>Intra-Run SD</th>
<th>Total SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Neg</td>
<td>108</td>
<td>0.0 %</td>
<td>2.07</td>
<td>0.00</td>
<td>0.00</td>
<td>1.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>2 Low pos</td>
<td>108</td>
<td>100 %</td>
<td>10.95</td>
<td>0.1</td>
<td>1.3</td>
<td>0.0</td>
<td>0.1</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>3 Low pos</td>
<td>108</td>
<td>100 %</td>
<td>9.78</td>
<td>0.4</td>
<td>4.1</td>
<td>1.3</td>
<td>12.9</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>4 Neg</td>
<td>107</td>
<td>100 %</td>
<td>1.94</td>
<td>0.0</td>
<td>0.0</td>
<td>2.2</td>
<td>0.0</td>
<td>0.8</td>
<td>0.1</td>
</tr>
<tr>
<td>5 Mod pos</td>
<td>108</td>
<td>100 %</td>
<td>9.07</td>
<td>0.3</td>
<td>3.7</td>
<td>0.4</td>
<td>4.5</td>
<td>0.6</td>
<td>6.7</td>
</tr>
<tr>
<td>6 Mod pos</td>
<td>108</td>
<td>100 %</td>
<td>15.44</td>
<td>0.1</td>
<td>0.6</td>
<td>0.0</td>
<td>0.0</td>
<td>1.4</td>
<td>8.8</td>
</tr>
<tr>
<td>7 Mod pos</td>
<td>108</td>
<td>100 %</td>
<td>22.15</td>
<td>0.4</td>
<td>1.7</td>
<td>0.6</td>
<td>2.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>8 Neg</td>
<td>106</td>
<td>0.0 %</td>
<td>2.04</td>
<td>0.0</td>
<td>0.5</td>
<td>0.2</td>
<td>2.4</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>9 Neg</td>
<td>107</td>
<td>97.2 %</td>
<td>2.02</td>
<td>0.0</td>
<td>0.7</td>
<td>0.1</td>
<td>2.7</td>
<td>0.0</td>
<td>1.3</td>
</tr>
<tr>
<td>10 Low pos</td>
<td>108</td>
<td>100 %</td>
<td>10.71</td>
<td>0.0</td>
<td>0.0</td>
<td>0.2</td>
<td>1.7</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>11 Low pos</td>
<td>108</td>
<td>100 %</td>
<td>9.43</td>
<td>0.4</td>
<td>4.4</td>
<td>0.7</td>
<td>7.2</td>
<td>0.4</td>
<td>4.0</td>
</tr>
<tr>
<td>12 Neg</td>
<td>108</td>
<td>100 %</td>
<td>2.08</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>2.3</td>
<td>0.0</td>
<td>0.9</td>
</tr>
<tr>
<td>13 Mod pos</td>
<td>108</td>
<td>100 %</td>
<td>11.18</td>
<td>0.2</td>
<td>1.5</td>
<td>0.1</td>
<td>1.2</td>
<td>0.0</td>
<td>0.2</td>
</tr>
<tr>
<td>14 Mod pos</td>
<td>108</td>
<td>100 %</td>
<td>11.63</td>
<td>0.0</td>
<td>0.0</td>
<td>0.5</td>
<td>3.9</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>15 Mod pos</td>
<td>108</td>
<td>100 %</td>
<td>22.97</td>
<td>0.5</td>
<td>2.2</td>
<td>0.2</td>
<td>0.9</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>16 Neg</td>
<td>108</td>
<td>100 %</td>
<td>1.93</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

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

^1 invalid reaction not retested

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

Cross-Reactivity

The analytical specificity of the APTIMA HPV Assay was evaluated with PreservCyt solution media diluted into APTIMA specimen transport media and spiked with cultured bacteria, yeast, or fungi; cultured virus; or low-risk HPV in vitro transcripts. The analytical sensitivity was evaluated with the same panel spiked with a low concentration of HPV infected SiHa cells (1 cell per reaction). The organisms and test concentrations are identified in Table 17. No effect on APTIMA HPV Assay specificity or sensitivity was observed with any of the organisms tested.

### Table 17: Analytical Specificity Panel

<table>
<thead>
<tr>
<th>Organism</th>
<th>Test Concentration</th>
<th>Organism</th>
<th>Test Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter lwoffii</td>
<td>1x10⁶ CFU/mL</td>
<td>Klebsiella pneumoniae</td>
<td>1x10⁶ CFU/mL</td>
</tr>
<tr>
<td>Actinomyces israelii</td>
<td>1x10⁶ CFU/mL</td>
<td>Lactobacillus acidophilus</td>
<td>1x10⁶ CFU/mL</td>
</tr>
<tr>
<td>Adenovirus 2</td>
<td>1x10⁶ vp/mL</td>
<td>Lactobacillus crispatus</td>
<td>1x10⁶ CFU/mL</td>
</tr>
<tr>
<td>Alcaligenes faecalis</td>
<td>1x10⁶ CFU/mL</td>
<td>Lactobacillus delbrueckii ssp. bulgaricus</td>
<td>1x10⁶ CFU/mL</td>
</tr>
<tr>
<td>Atopobium vaginae</td>
<td>5x10⁷ CFU/mL</td>
<td>Lactobacillus jensenii</td>
<td>1x10⁶ CFU/mL</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>1x10⁶ CFU/mL</td>
<td>Listeria monocytogenes</td>
<td>1x10⁶ CFU/mL</td>
</tr>
<tr>
<td>Bacteroides fragilis</td>
<td>1x10⁶ CFU/mL</td>
<td>Micrococcus luteus</td>
<td>1x10⁶ CFU/mL</td>
</tr>
</tbody>
</table>
Table 17: Analytical Specificity Panel (continued)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Test Concentration</th>
<th>Organism</th>
<th>Test Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacteroides ureolyticus</em></td>
<td>1x10^8 CFU/mL</td>
<td><em>Mobiluncus curtisii</em></td>
<td>2x10^7 CFU/mL</td>
</tr>
<tr>
<td><em>Bifidobacterium adolescentis</em></td>
<td>1x10^8 CFU/mL</td>
<td><em>Mycobacterium smegmatis</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>Bifidobacterium breve</em></td>
<td>1x10^8 CFU/mL</td>
<td><em>Mycoplasma fermentans</em></td>
<td>5x10^7 CFU/mL</td>
</tr>
<tr>
<td><em>Campylobacter fetus-fetus</em></td>
<td>1x10^8 CFU/mL</td>
<td><em>Mycoplasma genitalium</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>1x10^8 CFU/mL</td>
<td><em>Mycoplasma hominis</em></td>
<td>5x10^7 CFU/mL</td>
</tr>
<tr>
<td><em>Chlamydia trachomatis</em></td>
<td>2x10^8 TCID 50/mL</td>
<td><em>Neisseria gonorrhoeae</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>Clostridium difficile</em></td>
<td>6x10^7 CFU/mL</td>
<td><em>Neisseria gonorrhoeae</em> and</td>
<td>5x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>1x10^7 CFU/mL</td>
<td><em>Chlamydia trachomatis</em></td>
<td>1.5x10^4 TCID 50/mL</td>
</tr>
<tr>
<td><em>Corynebacterium genitalium</em></td>
<td>1x10^8 CFU/mL</td>
<td><em>Peptostreptococcus anaerobius</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>Corynebacterium xerosis</em></td>
<td>1x10^8 CFU/mL</td>
<td><em>Peptostreptococcus anaerobius</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>Cytomegalovirus</em></td>
<td>33 TCID 50/mL</td>
<td><em>Propionibacterium acnes</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>1x10^8 CFU/mL</td>
<td><em>Proteus mirabilis</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>1x10^8 CFU/mL</td>
<td><em>Proteus vulgaris</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>Epstein-Barr virus</em></td>
<td>4x10^7 vp/mL</td>
<td><em>Providencia stuartii</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>1x10^8 CFU/mL</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>Finegoldia magna</em></td>
<td>1x10^8 CFU/mL</td>
<td><em>Ruminococcus productus</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>Fusobacterium nucleatum</em></td>
<td>1x10^8 CFU/mL</td>
<td><em>Serratia marcescens</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>Gardnerella vaginalis</em></td>
<td>1x10^8 CFU/mL</td>
<td><em>Staphylococcus aureus</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>Haemophilus ducreyi</em></td>
<td>1x10^8 CFU/mL</td>
<td><em>Staphylococcus epidermidis</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>Herpes simplex virus 1</em></td>
<td>2.5x10^8 TCID 50/mL</td>
<td><em>Staphylococcus saprophyticus</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>Herpes simplex virus 2</em></td>
<td>5x10^8 TCID 50/mL</td>
<td><em>Streptococcus agalactiae</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>HIV-1</em></td>
<td>1.0x10^6 copies/mL</td>
<td><em>Streptococcus pyogenes</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>HPV 6</em></td>
<td>2.5x10^6 copies/mL</td>
<td><em>Streptococcus sanguinis</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>HPV 11</em></td>
<td>2.5x10^6 copies/mL</td>
<td><em>SV40</em></td>
<td>1.2 x 10^6 TCID 50/mL</td>
</tr>
<tr>
<td><em>HPV 42</em></td>
<td>2.5x10^6 copies/mL</td>
<td><em>Trichomonas vaginalis</em></td>
<td>1x10^7 cells/mL</td>
</tr>
<tr>
<td><em>HPV 43</em></td>
<td>2.5x10^6 copies/mL</td>
<td><em>Ureaplasma urealyticum</em></td>
<td>1x10^6 CFU/mL</td>
</tr>
<tr>
<td><em>HPV 44</em></td>
<td>2.5x10^6 copies/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>HPV 53</em></td>
<td>2.5x10^6 copies/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>HPV 61</em></td>
<td>2.5x10^6 copies/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>HPV 71</em></td>
<td>2.5x10^6 copies/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>HPV 81</em></td>
<td>2.5x10^6 copies/mL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Interference

The substances described in Table 18 were individually spiked into PreservCyt solution and APTIMA Specimen Transport Media (STM) at 1% and 10% v/v or w/v and tested with the APTIMA HPV Assay. All substances were tested in the presence and absence of HPV infected cultured cells (SiHa, 3 cells/reaction). Interference was not observed with any of the substances tested, except with two of the seven lubricants that contained Polyquaternium 15 at concentrations > 0.025% in the test sample, and an anti-fungal medication containing tioconazole at concentrations > 0.075% in the test sample.

Table 18: Substances Tested for Possible Interference with the APTIMA HPV Assay

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

*Personal lubricants that contain Polyquaternium 15.
PANTHER System Assay Performance

Clinical Panel Agreement Study

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 Cervical Specimen Collection and Transport (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 is shown in Table 19.

Agreement between the PANTHER and TIGRIS DTS Systems was > 98%, as shown in Table 19. 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% C.I: 92.2-99.7) on the TIGRIS DTS system. Sensitivity for detection of CIN3+ was 100% (C.I: 90.8%-100%) on both PANTHER and TIGRIS DTS systems.

Table 19: Agreement of APTIMA HPV Assay results from APTIMA Cervical Specimen Collection and Transport (CSCT) specimens tested on the TIGRIS DTS and PANTHER Systems

<table>
<thead>
<tr>
<th>TIGRIS DTS System</th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PANTHER System</td>
<td>+</td>
<td>490</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>9</td>
<td>130</td>
</tr>
<tr>
<td>Total</td>
<td>499</td>
<td>133</td>
<td>632</td>
</tr>
</tbody>
</table>

Overall Agreement = 98.1% (CI 96.7-98.9)  
Positive Agreement = 98.2% (CI 96.6-99.0)  
Negative Agreement = 97.7% (CI 93.9-99.2)

Analytical Sensitivity

The analytical sensitivity of the APTIMA HPV Assay was determined using a negative specimen matrix made by spiking C33A cervical carcinoma cells at 1000 cells/rxn (2500 cells/ml) in APTIMA Specimen Transport Media (STM). High risk HPV in-vitro transcripts were individually spiked into this matrix at 6 concentrations. These were tested using two reagent lots on multiple PANTHER Systems for a total of sixty replicates for each copy level. Probit regression analysis was performed and the predicted 95% detection limit determined for each HPV type (Table 20).

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

Table 20: Predicted 95% Detection Limit of the APTIMA HPV Assay Determined by Probit Analysis of PANTHER System Data

| Target | 95% Detection Limit*  
<table>
<thead>
<tr>
<th></th>
<th>(95% Fiducial Limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV 16</td>
<td>50 (37 - 77)</td>
</tr>
<tr>
<td>HPV 18</td>
<td>14 (11 - 21)</td>
</tr>
<tr>
<td>HPV 31</td>
<td>20 (15 - 30)</td>
</tr>
<tr>
<td>HPV 33</td>
<td>24 (19 - 34)</td>
</tr>
<tr>
<td>HPV 35</td>
<td>23 (17 - 36)</td>
</tr>
<tr>
<td>HPV 39</td>
<td>21 (15 - 36)</td>
</tr>
<tr>
<td>HPV 45</td>
<td>19 (14 - 28)</td>
</tr>
</tbody>
</table>
Assay Reproducibility

APTIMA HPV Assay reproducibility was evaluated on the PANTHER System using a 9-member panel comprised of negative, low positive (~95% detection level), and moderate positive panel members (~3x the 95% detection level). Positive panel members were comprised of STM spiked with HPV infected cultured cell lines (SiHa, HeLa, ME180, and MS751) and in-vitro transcript (HPV 16 and HPV 18). Negative panel members were comprised of STM and a Cervical Specimen Collection Transport (CSCT) specimen pool. The reproducibility panel is described in Table 21.

Testing was performed on 3 PANTHER Systems by 3 operators using 2 lots of reagents. All panel members were tested in triplicate per assay run, and testing was conducted over 10 days. One-hundred and eight data points for each reproducibility panel member were analyzed for the PANTHER System. The results are summarized in Table 22.

The percent positive for the negative panels ranged from 0 to 1.9%; low positive was ≥ 96%; and the moderate positive was 100%. The agreement with the expected result was > 96% for all the panel members.

The mean internal control (IC) S/CO was determined for the 2 negative panel members (1 and 8); the inter-instrument, -lot, and -run variability was calculated, as well as the intra-run variability. The mean IC S/CO for the negative panel members ranged from 1.77 to 2.01. The coefficient of variation (CV) for the IC S/CO values was < 9% for all parameters evaluated. The variability of the analyte S/CO values for the negative panel members was not analyzed due to the inherent variability when values of zero are observed.

The mean analyte S/CO was determined for the seven positive panel members (2, 3, 4, 5, 6, 7, and 9). The inter-instrument, - lot, and -run variability was calculated, as well as the intra-run variability.

### Table 21: PANTHER System APTIMA HPV Assay Reproducibility Panel

<table>
<thead>
<tr>
<th>Panel Member</th>
<th>Description</th>
<th>Concentration</th>
<th>Expected HPV Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CSCT Clinical Pool</td>
<td>N/A</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>SiHa Low Positive</td>
<td>1 cell/rxn</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>HPV 16 / HPV 18 Co-Infected Moderate Positive</td>
<td>100/100 copies/rxn</td>
<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>HPV 18 IVT Moderate Positive</td>
<td>100 copies/rxn</td>
<td>Positive</td>
</tr>
<tr>
<td>5</td>
<td>ME180 Moderate Positive</td>
<td>1 cell/rxn</td>
<td>Positive</td>
</tr>
<tr>
<td>6</td>
<td>MS751 Moderate Positive</td>
<td>1 cell/rxn</td>
<td>Positive</td>
</tr>
<tr>
<td>7</td>
<td>HPV 16 IVT Moderate Positive</td>
<td>100 copies/rxn</td>
<td>Positive</td>
</tr>
<tr>
<td>8</td>
<td>STM</td>
<td>N/A</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>HeLa Low Positive</td>
<td>0.15 cell/rxn</td>
<td>Positive</td>
</tr>
</tbody>
</table>

*copies per reaction

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### Table 20: Predicted 95% Detection Limit of the APTIMA HPV Assay Determined by Probit Analysis of PANTHER System Data (continued)

| HPV 51 | 63 (46 - 108) |
| HPV 52 | 292 (223 - 424) |
| HPV 56 | 61 (47 - 90) |
| HPV 58 | 60 (45 - 90) |
| HPV 59 | 84 (64 - 122) |
| HPV 66 | 49 (36 - 76) |
| HPV 68 | 31 (23 - 49) |
The mean analyte S/CO values ranged from 9.94 to 11.43 for the low positive panels and 8.50 to 20.56 for the moderate positive panels. The three panel members containing high-risk HPV types, panels 3, 4, and 7, had mean analyte S/CO values between 10.66 and 20.56. The CVs for the low positive and moderate positive panel members were < 30% and ≤ 23% respectively, with the highest variability observed within a run. The internal control S/CO values were not evaluated for the positive panel members because the internal control RLUs are not indicative of an individual reaction's performance in an analyte-positive sample.

Table 22: APTIMA HPV Assay Reproducibility on the PANTHER System

<table>
<thead>
<tr>
<th>Panel Member</th>
<th>Description</th>
<th>N</th>
<th>% Positive</th>
<th>Agreement</th>
<th>IC</th>
<th>Analyte</th>
<th>SD</th>
<th>CV (%)</th>
<th>Inter-Instrument</th>
<th>SD</th>
<th>CV (%)</th>
<th>Inter-Lot</th>
<th>SD</th>
<th>CV (%)</th>
<th>Inter-Run</th>
<th>SD</th>
<th>CV (%)</th>
<th>Intra-Run</th>
<th>SD</th>
<th>CV (%)</th>
<th>Total</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Neg</td>
<td>107</td>
<td>1.9</td>
<td>98.1%</td>
<td>N/A</td>
<td>1.77</td>
<td>0.05</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
<td>3.9</td>
<td>0.1</td>
<td>6.0</td>
<td>0.1</td>
<td>5.1</td>
<td>0.2</td>
<td>8.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Low Pos</td>
<td>108</td>
<td>96.3%</td>
<td>N/A</td>
<td>9.94</td>
<td>0.8</td>
<td>7.8</td>
<td>0.2</td>
<td>2.5</td>
<td>0.0</td>
<td>0.0</td>
<td>2.8</td>
<td>28.6</td>
<td>3.0</td>
<td>29.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Mod Pos</td>
<td>108</td>
<td>100.0%</td>
<td>N/A</td>
<td>20.56</td>
<td>0.5</td>
<td>7.8</td>
<td>0.9</td>
<td>4.2</td>
<td>1.6</td>
<td>8.0</td>
<td>2.1</td>
<td>10.1</td>
<td>2.9</td>
<td>14.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Mod Pos</td>
<td>108</td>
<td>100.0%</td>
<td>N/A</td>
<td>11.02</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.6</td>
<td>15.0</td>
<td>1.9</td>
<td>17.5</td>
<td>2.5</td>
<td>23.1</td>
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<td></td>
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</tr>
<tr>
<td>5</td>
<td>Mod Pos</td>
<td>108</td>
<td>100.0%</td>
<td>N/A</td>
<td>8.50</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.1</td>
<td>13.1</td>
<td>0.7</td>
<td>8.5</td>
<td>1.3</td>
<td>15.6</td>
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<td></td>
</tr>
<tr>
<td>6</td>
<td>Mod Pos</td>
<td>108</td>
<td>100.0%</td>
<td>N/A</td>
<td>12.81</td>
<td>0.0</td>
<td>0.0</td>
<td>0.8</td>
<td>6.6</td>
<td>1.1</td>
<td>8.7</td>
<td>1.5</td>
<td>11.6</td>
<td>2.2</td>
<td>16.8</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>7</td>
<td>Mod Pos</td>
<td>108</td>
<td>100.0%</td>
<td>N/A</td>
<td>10.66</td>
<td>0.1</td>
<td>0.7</td>
<td>0.0</td>
<td>0.0</td>
<td>0.3</td>
<td>2.5</td>
<td>0.3</td>
<td>3.2</td>
<td>0.5</td>
<td>4.1</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>8</td>
<td>Neg</td>
<td>108</td>
<td>0.0</td>
<td>100.0%</td>
<td>N/A</td>
<td>2.01</td>
<td>0.00</td>
<td>0.0</td>
<td>0.9</td>
<td>0.0</td>
<td>1.6</td>
<td>0.0</td>
<td>2.4</td>
<td>0.1</td>
<td>4.1</td>
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<td></td>
</tr>
<tr>
<td>9</td>
<td>Low Pos</td>
<td>108</td>
<td>100.0%</td>
<td>N/A</td>
<td>11.43</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.2</td>
<td>10.9</td>
<td>1.6</td>
<td>13.8</td>
<td>2.1</td>
<td>18.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*internal control S/CO variability analysis for the negative panels (1 and 8); analyte S/CO variability analysis for the positive panels (2, 3, 4, 5, 6, 7, 9)

1 invalid reaction not retested

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

IC = Internal Control

Analytical Specificity

Analytical specificity was evaluated using in vitro transcript of the low-risk HPV genotypes 6, 11, 42, 43, and 44. Transcripts for each low-risk type were spiked into STM at 2.5×10^6 copies/mL (1×10^6 copies/reaction). No effect on APTIMA HPV Assay results was observed with any of the genotypes tested on the PANTHER System.

Interference

Performance of the APTIMA HPV Assay on the PANTHER System in the presence of blood was evaluated with and without high-risk HPV present in STM. No interference was observed in the presence of whole blood present at 10% (volume/volume).


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