**PROCEDURE: CORO MOLECULAR MICROBIOLOGY APTIMA HPV 16, 18/45 GENOTYPE ASSAY (PANTHER)**

1. **PRINCIPLE**
   1. The APTIMA HPV 16 18/45 Genotype Assay is an in vitro nucleic acid amplification test for the qualitative detection of E6/E7 viral messenger RNA (mRNA) of human papillomavirus (HPV) types 16, 18, and 45 in cervical specimens from women with APTIMA HPV Assay positive results. The APTIMA HPV 16 18/45 Genotype Assay can differentiate HPV 16 from HPV 18 and/ or HPV 45, but does not differentiate between HPV 18 and HPV 45. Cervical specimens in ThinPrep Pap Test vials containing PreservCyt Solution and collected with broom-type or cytobrush/spatula collection devices may be tested with the APTIMA HPV 16 18/45 Genotype Assay. The assay is used with the PANTHER System.
   2. The APTIMA HPV 16 18/45 Genotype Assay involves three main steps, which take place in a single tube: target capture; target amplification by Transcription-Mediated Amplification (TMA); and detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA). The assay incorporates an Internal Control (IC) to monitor nucleic acid capture, amplification, and detection, as well as operator or instrument error.
   3. Specimens are transferred to a tube containing specimen transport media (STM) that lyses the cells, releases the mRNA, and protects it from degradation during storage. When the APTIMA HPV 16 18/45 Genotype Assay is performed, the target mRNA is isolated from the specimen by use of capture oligomers that are linked to magnetic microparticles. The capture oligomers contain sequences complementary to specific regions of the HPV mRNA target molecules as well as a string of deoxyadenosine residues. During the hybridization step, the sequence-specific regions of the capture oligomers bind to specific regions of the HPV mRNA target molecule. The capture oligomer-target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles, including the captured HPV mRNA target molecules bound to them, are pulled to the side of the reaction tube using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification inhibitors.
   4. 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.
   5. Detection of the amplicon is achieved by HPA using single-stranded nucleic acid probes with chemiluminescent labels that are complementary to the amplicon. The labeled nucleic acid probes hybridize specifically to the amplicon. The Selection Reagent differentiates between hybridized and unhybridized probes by inactivating the label on the unhybridized probes. During the detection step, light emitted from the labeled RNA: DNA hybrids is measured as photon signals called Relative Light Units (RLU) in a luminometer. Final assay results are interpreted based on the analyte signal-to-cutoff (S/CO) ratio.
   6. IC is added to each reaction via the Target Capture Reagent. The IC monitors the target capture, amplification, and detection steps of the assay. The Dual Kinetic Assay (DKA) is the method used to differentiate the HPV signals and the IC signal. IC and HPV 16 amplicon are detected by probes with rapid light-emission kinetics (flasher). The IC signal in each reaction is discriminated from the HPV 16 signal by the magnitude of the light emission. Amplicons specific to HPV 18 and 45 are detected using probes with relatively slower kinetics of light emission (glower).
2. **AVAILIBILITY**
   1. Test is performed once per week on Wednesday. Specimens may be submitted 7 days/week, 24 hours/day.
3. **TEST CODE**
   1. HPVG1
      1. HPV Genotype can be ordered as a reflex test to HPV orders.
      2. HPV Genotype can be added on to an HPV Positive sample.
4. **SPECIMEN COLLECTION AND HANDLING**
   1. Specimen collection and processing:
      1. Collect cervical specimens in ThinPrep Pap Test vials containing PreservCyt Solution with broom-type or cytobrush/spatula collection devices according to the manufacturer’s directions.
      2. The ThinPrep Aliquot Removal procedure will be used where 1 ml of the PreservCyt Solution liquid cytology specimen will be removed before being processed in cytology and pipetted into an APTIMA Specimen Transfer Tube. Refer to Tomcat Operating Procedure and Checklist for directions and Appendix E of this procedure for manual transfer.
   2. Transport and storage before testing:
      1. Transport the ThinPrep liquid cytology specimens at 2°C to 30°C.
      2. PreservCyt specimens should be transferred to an APTIMA Specimen Transfer tube within 30 days of collection.
      3. Specimen Transfer tube may be stored at 2°C to 30°C for up to 60 days or -20°C for up to 24 months.
   3. Specimen storage after testing:
      1. Specimens that have been tested must be stored upright in a rack.
      2. Specimen tubes should be covered with a new, clean plastic or foil barrier.
      3. One rack of completed HPVG samples will be held at room temperature.
5. **EQUIPMENT AND MATERIALS**
   1. Equipment
      1. Panther System (Cat. No. 303095)
   2. Reagents and Materials Provided
      1. APTIMA HPV 16 18/45 Genotype Assay

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

* + - 1. 100 Tests. Cat. No. 303236 (3 boxes)
      2. Kit Components Aptima HPV 16 18/45 Genotype Assay Refrigerator Box

Note: Store at 2°C to 8°C upon receipt.

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

* + - 1. Kit Components Aptima HPV 16 18/45 Genotype Assay Room Temperature Box

Note: Store at 15°C to 30°C upon receipt.

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

* + - 1. APTIMA HPV 16 18/45 Genotype Assay Calibrators Box (Cat. No. 303235) Note: store at 2°C to 8°C upon receipt

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

* 1. Materials Required Available Separately

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

|  |  |
| --- | --- |
|  | **Cat. #** |
| PANTHER System Run Kit | 303096 |
| APTIMA Assay Fluids Kit  (APTIMA Wash Solution, APTIMA Buffer for Deactivation Fluid, and APTIMA Oil Reagent) | 303014 |
| APTIMA Auto Detect Kit | 303013 |
| Multi-tube units (MTUs) | 104772-02 |
| PANTHER Waste Bag Kit | 902731 |
| PANTHER Waste Bin Cover | 902714 |
| Tips, 1000 µL conductive, liquid sensing | 10612513 (Tecan) |
| APTIMA Specimen Transfer Kit | 301154C |
| APTIMA Penetrable Caps | 105668 |
| Replacement non-penetrable caps | 103036A |
| Spare Caps for 100 test kits: |  |
| Amplification Reagent and Probe Reagent reconstitution solutions | CL0041 |
| Enzyme Reagent reconstitution solution | CL0041 |
| TCR and Selection Reagent | 501604 |
| Bleach, minimum 5% or 0.7 M sodium hypochlorite solution | Stores item |
| Disposable gloves | Stores item |
| AcroMetrix Negative Control | 950078 |
| AcroMetrix Positive Control HPV GT 16 | 950075 |
| AcroMetrix Positive Control HPV GT 18 | 950076 |

* 1. Reagent Storage and Handling Requirements
     1. The following reagents are stable when stored at 2⁰C to 8⁰C (refrigerated):
        1. APTIMA HPV 16, 18/45 Amplification Reagent
        2. APTIMA HPV 16, 18/45 Enzyme Reagent
        3. APTIMA HPV 16, 18/45 Probe Reagent
        4. APTIMA HPV 16, 18/45 Internal Control Reagents
        5. APTIMA HPV 16, 18/45 Positive and Negative Calibrators
     2. The following reagents are stable when stored at 15°C to 30°C (room temperature):
        1. APTIMA HPV 16, 18/45 Amplification Reconstitution Solution
        2. APTIMA HPV 16, 18/45 Enzyme Reconstitution Solution
        3. APTIMA HPV 16, 18/45 Probe Reconstitution Solution
        4. APTIMA HPV 16, 18/45 Target Capture Reagent
        5. APTIMA HPV 16, 18/45 Selection Reagent
        6. Wash Solution
        7. Oil Reagent
        8. Buffer for Deactivation
        9. Auto Detect Reagent 1
        10. Auto Detect Reagent 2
     3. After reconstitution, Enzyme Reagent, Amplification Reagent, and Probe Reagent are stable for 30 days when stored at 2⁰C to 8⁰C
     4. Working Target Capture Reagent (wTCR) is stable for 30 days when stored at 15°C to 30°C. Do not refrigerate.
     5. Discard any unused reconstituted reagents and wTCR after 30 days, or after the Master Lot expiration date, whichever comes first.
     6. The Aptima HPV 16 18/45 Genotype Assay reagents are stable for a cumulative of 72 hours when stored on board the PANTHER System.
     7. Avoid cross-contamination during reagent handling and storage. Recap all reconstituted reagents with new reagent caps each time prior to storage.
     8. The Probe Reagent and Reconstituted Probe Reagent are photosensitive. Store the reagents protected from light.
     9. Do not freeze reagents

1. **Warnings and Precautions**
   1. For in vitro diagnostic use.
   2. For additional specific warnings and precautions related to instrumentation refer to the PANTHER System Operator’s Manual.
   3. Laboratory Related
      1. Use only supplied or specified disposable laboratory ware.
      2. Use routine laboratory precautions. Do not eat, drink or smoke in designated work areas. Wear disposable, powder less gloves, and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.
      3. 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 with water. If these fluids spill, dilute the spill with water before wiping dry.
      4. 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.
   4. Specimen Related
      1. Test only the indicated specimen type. The APTIMA HPV 16 18/45 Genotype Assay has only been validated for use with cervical specimens collected in PreservCyt Solution using a broom- type or cytobrush/spatula collection device.
      2. Collect cervical specimens in ThinPrep Pap Test vials containing PreservCyt Solution with broom- type or cytobrush/spatula collection devices according to the manufacturer’s instructions. Aliquots subsequently removed from the ThinPrep Pap Test vial for testing with the APTIMA HPV 16 18/45 Genotype Assay should be processed using only the APTIMA Specimen Transfer Kit.
      3. ThinPrep liquid cytology specimens were evaluated for use with the APTIMA HPV 16 18/45 Genotype Assay after processing on the ThinPrep 2000 System. Specimens processed using the ThinPrep 3000 System or other instruments have not been evaluated.
      4. Maintain proper temperature conditions during specimen shipping and storage to ensure the integrity of the specimen. Specimen stability has not been evaluated under shipping and storage conditions other than those recommended.
      5. Expiration dates listed on specimen transfer kits and tubes pertain to the transfer site and not the testing facility. Specimens transferred any time prior to these expiration dates are valid for testing provided they have been transported and stored in accordance with the appropriate package insert, even if these expiration dates have passed.
      6. 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.
      7. 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.
      8. Liquid can discharge from tube caps upon piercing under certain conditions. Refer to the PANTHER System Test Procedure for more information.
   5. Assay Related
      1. Store reagents at the specified temperatures. Performance of the assay may be affected by use of improperly stored reagents.
      2. Avoid microbial and ribonuclease contamination of reagents.
      3. Do not use kit after its expiration date.
      4. Do not interchange, mix, or combine assay reagents or Calibrators from kits with different lot numbers.
      5. APTIMA Assay Fluids and APTIMA Auto Detect Reagents are not part of the Master Lot; any lot may be used.
      6. Thorough mixing of assay reagents is necessary to achieve accurate assay results.
      7. Tips with hydrophobic plugs must be used.
2. **TEST PROCEDURE**

**Note:** See **PANTHER System Operator’s Manual** for additional Panther System

procedural information. Refer to the complete Panther System Operator’s Manual

located in the software of the Panther System and on the “K” drive for additional

procedural information.

For quick reference guides refer to Appendix A: Panther System Operation Checklist

and Appendix B: Panther Resources Needed located at the end of this procedure.

* 1. Laboratory/ Panther Preparation (Daily)
     1. Prior to starting the assay, wipe down work surfaces with household bleach diluted 1:1 with water (1 part bleach, 1 part water). Allow bleach to contact surfaces for at least 1 minute and then follow with a DI water rinse. Do not allow the bleach to dry. Cover the bench surface on which the test will be performed with clean, plastic-backed absorbent laboratory bench covers.
     2. \*\*\*CHANGE GLOVES
     3. Remove reagent kits from refrigerator and bring to room temp (30 Min). If a new kit needs to be made it should be done at this point. See the REAGENT RECONSTITUTION/PREPERATION section of this procedure.
  2. Reagent Reconstitution/ Preparation

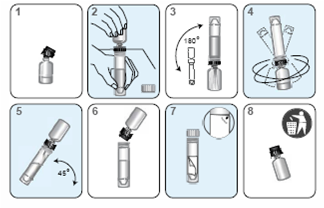
**Note:** This step should be performed prior to beginning any work on the PANTHER

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.
       1. 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.
       2. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
       3. Open the lyophilized reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 1, Step 1).
       4. Open the matching reconstitution solution bottle and set the cap on a clean, covered work surface.
       5. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 1, Step 2).
       6. Slowly invert the assembled bottle and vial. Allow the solution to drain from the bottle into the vial (Figure 1, Step 3).
       7. Gently swirl the solution in the vial to mix. Avoid creating foam while swirling the vial (Figure 1, Step 4).
       8. Wait for the lyophilized reagent to go into solution, then invert the assembled bottle and vial again, tilting at a 45° angle to minimize foaming (Figure 1, Step 5). Allow all of the liquid to drain back into the bottle.
       9. Remove the reconstitution collar and glass vial (Figure 1, Step 6).
       10. Recap the bottle. Record operator initials and the reconstitution date. (Figure 1, Step 7).
       11. Discard the reconstitution collar and vial (Figure 1, Step 8).

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

**Figure 1**



* + 1. Prepare Working Target Capture Reagent (wTCR)
       1. Pair the appropriate bottles of TCR and TCR-B.
       2. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.
       3. Open the bottle of TCR, and set the cap on a clean, covered work surface.
       4. Open the bottle of TCR-B and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the TCR-B bottle.
       5. Cap the bottle of TCR and gently swirl the solution to mix the contents. Avoid creating foam during this step.
       6. Record operator initials and the current date on the label.
       7. Discard the TCR-B bottle and cap.
    2. Prepare Selection Reagent
       1. Check the lot number on the reagent bottle to make sure it matches the lot number on the Master Lot Barcode Sheet.
       2. Record operator initials and the current date on the label.
    3. Thoroughly mix by gently inverting all reagents prior to loading on the system. Avoid creating foam during inversion of reagents.
  1. 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 the reconstituted Probe Reagent contains a precipitate that does not return to solution at room temperature, heat the capped bottle at a temperature that does not exceed 62°C for 1 to 2 minutes. Do not use if precipitate or cloudiness persist.
     3. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents.
     4. Do not top off reagent bottles. The PANTHER System will recognize and reject bottles that have been topped off.
     5. 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.
     6. If the Selection Reagent contains precipitate, warm the Selection Reagent at 60°C ± 1°C for up to 45 minutes to facilitate dissolution of precipitate. Gently mix the bottle every 5 to 10 minutes. Allow the Selection Reagent to equilibrate to room temperature prior to use. Do not use if precipitate or cloudiness persists.
     7. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents.
     8. Do not top off reagent bottles. The PANTHER System will recognize and reject bottles that have been topped off.
  2. Sample Handling
     1. Allow the samples (calibrators, specimens, and any user provided external control samples) to reach room temperature prior to processing.
     2. Do not vortex specimens.
     3. Inspect sample tubes before loading into the racks. If a sample tube contains bubbles or has a lower volume than is typically observed, centrifuge the tube for 5 minutes at 420 RCF to ensure that no liquid is in the cap.
     4. Note: Failure to follow step 3 may result in liquid discharge from the sample tube cap.
  3. System Preparation
     1. Set up the system and worklist according to the instructions in the Panther System Operator’s Manual and the Procedural Notes section below. Make sure that the appropriate sized reagent racks and TCR adapters are used.

1. **PROCEDURAL NOTES**
   1. Calibrators
      1. To work properly with the APTIMA 16 18/45 Genotype Assay software on the PANTHER System, two replicates of the Negative Calibrator and each Positive Calibrator are required. One vial of each calibrator may be loaded in any rack position in a Sample Bay Lane on the PANTHER System. Specimen pipetting will begin when one of the following two conditions has been met:
         1. Positive and Negative Calibrators are currently being processed by the PANTHER System.
         2. Valid results for the calibrators are registered on the PANTHER System.
      2. Once the calibrator tubes have been pipetted and are being processed for a specific reagent kit, specimens can be run with the associated assay reagent kit for up to 24 hours unless:
         1. Calibrators are invalid.
         2. The associated assay reagent kit is removed from the PANTHER System.
         3. The associated assay reagent kit has exceeded the stability limits.
      3. Attempts to pipette more than two replicates from a calibrator tube can lead to insufficient volume errors.
   2. Temperature
      1. Room temperature is defined as 15°-30°C.
   3. Glove Powder
      1. As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.
2. **QUALITY CONTROL** 
   1. Run Validity Criteria
      1. The software automatically determines run validity. The software will invalidate a run if any of the following conditions occur:
         1. More than one invalid Negative Calibrator replicate.
         2. More than one invalid Positive Calibrator 1 replicate.
         3. More than one invalid Positive Calibrator 2 replicate.
         4. More than 1 of 6 invalid calibrator replicates combined.
      2. A run may be invalidated by an operator if technical, operator, or instrument difficulties are observed and documented while performing the assay.
      3. An invalid run must be repeated. Aborted runs must be repeated.

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

* + 1. External quality control samples (not provided) should be tested in conformance with local, state, and/or federal regulations or accreditation requirements and each laboratory’s standard Quality Control procedures.
       1. External Positive and Negative controls are purchased from Acrometrix and are run each day of testing.
  1. Calibrator Acceptance Criteria
     1. The table below defines the RLU criteria for the Negative and Positive Calibrator replicates.

|  |  |
| --- | --- |
|  | PANTHER System |
| Negative Calibrator  18/45 RLU IC/16 RLU | *>=* 0 and <= *60,000* RLU  >=75,000 and <=*300,000* RLU |
| Positive Calibrator 1  18/45 RLU IC/16 RLU | *>=* 800,000 and <=*2,200,000* RLU  *<=* 475,000 RLU |
| Positive Calibrator 2  18/45 RLU IC/16 RLU | <=115,000 RLU  >=625,000 and <=4,000,000 RLU |

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

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

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

**Analyte 16 Cutoff=** 2x (meanIC/16 RLU of the valid Negative Calibrator

replicates) + 0.1(mean IC/16 RLU of the valid Positive Calibrator 2 replicates)

* 1. Analyte 16 Signal to Cutoff (S/CO)
     1. The analyte S/CO for HPV 16 is determined from the IC/16 RLU signal of the test sample and the analyte 16 cutoff for the run.

Test sample IC/16 RLU

**Analyte 16 S/CO=**analyte 16 cutoff

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

**Analyte 18/45 Cutoff=** 1x (mean18/45 RLU of the valid Negative Calibrator

replicates) + 0.18x (mean I8/45 RLU of the valid Positive Calibrator 1 replicates)

* 1. Analyte 18/45 Signal to Cutoff (S/CO)
     1. The analyte S/CO for HPV 18/45 is determined from the 18/45 RLU signal of the test sample and the analyte 18/45 cutoff for the run.

Test sample 18/45 RLU

**Analyte 18/45 S/CO=** analyte 18/45 cutoff

* 1. Control Acceptance Criteria
     1. The Negative Control must have a valid negative result for HPV 16 and HPV 18/45 (S/CO<1.00)
     2. The Positive HPV 16 Control must have a valid positive result for HPV 16 (S/CO>=1.00) and a valid negative result for HPV 18/45 (S/CO<1.00)
     3. The Positive HPV 18 Control must have a valid positive result for HPV 18/45 (S/CO>=1.00) and a valid negative result for HPV 16 (S/CO<1.00)
     4. Each new reagent lot/ new shipment of HPV 16, 18/45 Genotype kits must be QC’d with previously tested Positive and Negative External Controls:

AcroMetrix® HPV Genotype 16 and 18 Positive Controls and Negative Control

* + 1. New reagent lots/ new shipments of External Positive and Negative Controls must be tested concurrently with previously tested External Positive and Negative Control. The New Controls should be run as patient specimens with the Controls already in use.

1. **TEST INTERPRETATION**
   1. Test results are automatically determined by the assay software. A test result may be negative for both HPV 16 and HPV 18/45, negative for HPV 16 and positive for HPV 18/45, positive for HPV 16 and negative for HPV 18/45, positive for both HPV 16 and HPV 18/45, or invalid as determined by the RLU and S/CO ratios as described in the table below. A test result may also be invalid due to other parameters (e.g., abnormal curve shape) being outside the normal expected ranges. Invalid test results should be repeated.

|  |  |
| --- | --- |
| APTIMA HPV 16 18/45  Genotype Assay  Result | Criteria |
| Negative - 16  Negative - 18/45 | IC/HPV 16 RLU *>=* IC Cutoff and  HPV 16 S/CO < 1.00 and  HPV 18/45 S/CO < 1.00 |
| Negative - 16  Positive - 18/45 | HPV 16 S/CO < 1.00 an*d*  HPV 18/45 S/CO *>=* 1.00 and HPV 18/45 RLU <=3,000,000 |
| Positive - 16  Negative - 18/45 | HPV 16 S/CO *>=* 1.00 and  IC/HPV 16 RLU <=4,000,000 and  HPV 18/45 S/CO < 1.00 |
| Positive - 16  Positive - 18/45 | HPV 16 S/CO *>=* 1.00 and  IC/HPV 16 RLU <=4,000,000 and  HPV 18/45 S/CO *>=* 1.00 and  HPV 18/45 RLU <=3,000,000 |
| Invalid | HPV 16 S/CO < 1.00 and  HPV 18/45 S/CO < 1.00 and IC/HPV 16 RLU < IC cutoff  or  IC/HPV 16 RLU > 4,000,000  or  HPV 18/45 RLU > 3,000,000 |

1. **REPORTING PATIENT TEST RESULTS**

Refer to Appendix C for Soft Resulting

* 1. If the controls in any run do not yield the expected results, test results on patient specimens in the same run must not be reported.
  2. PreservCyt Solution HPV results
     1. Negative for HPV Genotype 16

Negative for HPV Genotype 18/45

* + 1. Negative for HPV Genotype 16

Positive for HPV Genotype 18/45

* + 1. Positive for HPV Genotype 16

Negative for HPV Genotype 18/45

* + 1. Positive for HPV Genotype 16

Positive for HPV Genotype 18/45

* + 1. Invalid- Invalid samples are retested.
       1. Invalid results that repeat as Invalid due to Volume Verification Failure Sample (VVFS) are resulted as @UNAZ- Unable to analyze due to sampling issues caused by specimen integrity. Please resubmit if clinically warranted.
       2. Invalid results that repeat as Invalid due to any other issue are resulted as Indeterminate with a texted comment stating the reason.
    2. Indeterminate
    3. Result Comment
       1. The following comment is added to all results:

“Assay is FDA-cleared for cervical specimens in Thin Prep vials collected with either a broom type device or endocervical brush.”

* + 1. All HPVG1 orders on HPV HR Negative samples will be autocanceled in Soft.
       1. HPV Genotype NOT PERFORMED

The HPV Genotype was not performed because results of the High Risk HPV test were not Positive.

1. **LIMITATIONS OF THE PROCEDURE:**
   1. The performance of the APTIMA HPV 16 18/45 Genotype Assay has

not been evaluated for HPV vaccinated individuals.

* 1. The APTIMA HPV 16 18/45 Genotype Assay has not been evaluated

in cases of suspected abuse.

* 1. Prevalence of HPV infection in a population may affect performance.

Positive predictive values decrease when testing populations with low prevalence or individuals with no risk of infection.

* 1. ThinPrep liquid cytology specimens containing less than 1 mL after

ThinPrep Pap Test slide preparation are considered inadequate for the APTIMA

HPV 16 18/45 Genotype Assay.

* 1. APTIMA HPV 16, 18/45 Genotype Assay performance has not been

evaluated with post- processed ThinPrep liquid cytology specimens using processors other than the ThinPrep 2000 System.

* 1. Test results may be affected by improper specimen collection,

storage, or specimen processing.

* 1. The Internal Control monitors the target capture, amplification, and

detection steps of the assay. It is not intended to control for cervical sampling adequacy.

* 1. A negative APTIMA HPV 16 18/45 Genotype Assay result does not

exclude the possibility of cytologic abnormalities or of future or underlying

CIN2, CIN3, or cancer.

* 1. The APTIMA HPV 16 18/45 Genotype Assay provides qualitative

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

* 1. Detection of high-risk HPV (types 16, 18, and 45) mRNA is dependent on the number of copies present in the specimen and may be affected by specimen collection methods, patient factors, stage of infection and the presence of interfering substances.
  2. Infection with HPV is not an indicator of cytologic HSIL or underlying

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

* 1. The following may interfere with the performance of the assay when

present at concentrations greater than those specified: vaginal lubricants (containing Polyquaternium 15) at 1% w/v, anti- fungal cream (containing tioconazole) at 0.03% w/v, mucus at 0.3% w/v, intravaginal hormones (containing progesterone) at 1% w/v, Trichomonas vaginalis at 3 x 104 cells/mL.

* 1. High concentrations of HPV 45 can reduce the ability of the APTIMA

HPV 16 18/45 Genotype Assay to detect the presence of HPV 16 at low levels.

* 1. The effects of other potential variables such as vaginal discharge, use

of tampons, etc. and specimen collection variables have not been evaluated.

* 1. Use of this device must be limited to personnel trained in the use of

the APTIMA HPV 16 18/45 Genotype Assay.

* 1. Cross-contamination of samples can cause false positive results. The carryover rate of the APTIMA HPV 16 18/45 Genotype Assay on the PANTHER System was 0.35% and 0.19% respectively, as determined in non-clinical studies.
  2. The APTIMA HPV 16 18/45 Genotype Assay should be interpreted in

conjunction with other laboratory and clinical data available to the clinician.

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* 1. Aptima HPV 16 18/45 Genotype Assay Package Insert

1. **TECHNICAL SUPPORT**
   1. Hologic, Inc.

10210 Genetic Center Drive

San Diego, CA 92121 USA

* 1. Customer Support
     1. 1 800 442 9892
     2. customersupport@hologic.com
  2. Technical Support
     1. 1 888 484 4747
     2. molecularsupport@hologic.com
  3. Additional contact information
     1. [www.hologic.com](http://www.hologic.com)

1. **REVISIONS**
   1. March 2019
      1. Updated format of procedure to Coro Molecular Microbiology
      2. Added Appendixes to Procedure
      3. Added HPVG autocancelling of HPV negative specimens in Appendix D and Results section.
      4. Deleted Canceling of HPVG orders on Negative HPV specimens from Appendix D
      5. Added the following comment to all results:

“Assay is FDA-cleared for cervical specimens in Thin Prep vials collected with either a broom type device or endocervical brush.”

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**Appendix A**

**Panther System Operation Checklist**

1. **Clean and Inspect**

* Clean work areas with 50% bleach (1 min) and rinse with DI H2O

**CHANGE GLOVES**

* Check room humidity (20%-85%)
* Check room temperature (15°-30°C)
* Perform external inspection
* Check for leaks
* Fill DI H2O bucket

**CHANGE GLOVES**

1. **Prepare Reagents**

* Take out reagent kits and bring to room temp (approx. 30 min.)
* Print Assay Reagents report (if needed)
* Reconstitute reagents, if necessary
* If using previously reconstituted reagents, bring reagents to room temp and gently invert all reagents
* Warm probe reagent, if necessary, and invert.

**CHANGE GLOVES**

1. **Complete Panther System Tasks**

* Load Tips
* Load MTUs

Check for:

* All 5 tiplets present
* No extra MTU tiplets inside tubes
* Barcodes present, properly aligned, undamaged, and intact.
* Distributor feet intact
* Maximum of 125 MTUs loaded
* Load Universal Fluids
* Replace depleted fluids
* Ensure all fittings are secure
* Empty Waste
* Ensure all fittings are secure

**CHANGE GLOVES**

* Perform Maintenance- Tasks
* Complete scheduled maintenance tasks
* Prime, if necessary
* Ensure resources are available
* Load Assay Reagents
* Remove caps, ensure no bubbles and/or precipitate is present
* Ensure bottles are properly seated
* Verify all barcodes are visible
* Activate New Lots of Reagents (if needed)
* Load onto the Panther System
* Ensure wTCR quadrant matches Assay Reagent lane for Amp, Enzyme, Probe, and Selection
* Load Samples
* Ensure sample default settings are accurate or test orders are available via LIS
* Rack calibrators and controls for each Assay Reagent kit loaded
* Confirm correct sample collection and volume
* Place samples into racks
* Verify all barcodes are visible
* Ensure sample retainers are seated
* Load onto the Panther System
* Check racks for any errors
* Manually enter order numbers if necessary
* Manually add test orders when necessary

**CHANGE GLOVES**

1. **Pipetting and Assay Processing**

Sample tube graphic will change from:

* Green- Sample loaded
* Yellow- Pipetting in-process
* Blue- Pipetting complete
* Red- Error

1. **Feed and Monitor**

* Return as needed to load tips, MTUs, additional sample racks, and Assay Reagents

**CHANGE GLOVES**

1. **Review/Manage Results**

* Print Results Report
* Print Exceptions Report
* Verify results
* Send selected results to LIS

1. **Unload Sample Racks**

* Remove sample racks
* Rack samples and cover with parafilm

**CHANGE GLOVES**

1. **Unload Reagents, if necessary**

* Recap with new caps and store Assay Reagents
* Deactivate any lots when all reagents have been used up.

1. **Check Fluid and Waste**

* Replenish/ Empty as needed

**Appendix B**

**Panther Resources Needed**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Full Prime** | **Mini-Prime** |  | **Mag Wash Clean Maintenance** |
| **Time to Complete** | 12 Minutes | 10 Minutes |  |  |
| **MTU Count Consumed** | 50/ 10 strips | 15/ 3 strips |  | 10/ 2 strips |
| **Waste Count Consumed** | 50 | 15 |  | 20 |
| **Maximun Fluid Test Count consumed per Kit** | 105 | 8 |  | 15 in Fluids A **OR** B |
| Mag Wash Cleaning Solution |  |  |  | 25.5 ml |

**Appendix C**

**HPV 16 18/45 Genotyping Panther Procedure Notes and SCC Soft Resulting**

Test ID: **HPVG1**

Template: **HPVG**

Workstation: **RMOLM**

1. Create a Tasklist
2. Follow procedure for “Creating a Tasklist” in Soft Manual under TASKLIST

Template= HPVG

1. Delete the calibrator and controls from the list
2. Wand specimens onto the tasklist beginning in position 1.
3. Load Assay Reagents onto Panther
4. Load HPVG Calibrators and External Controls into Sample Rack- Load onto Panther

**Note:** Label the HPVG controls with external control barcode labels.

1. Load HPVG sample tubes into sample Rack- Load onto Panther

Verify that sample barcodes and test orders were read.

1. Results
2. Panther Reports- Report Tab
3. Select “Results by Worklist” Report
4. Choose Worklist ID by Date and Assay
5. Print
6. Select “Exceptions by Worklist” Report

**Note:** List of all Positive and Invalid results

1. Choose Worklist ID by Date and Assay
2. Print
3. Select “Worklist Lot” Report
4. Choose Worklist ID by Date and Assay
5. Print
6. Check reports for any invalid samples, samples that need repeating, and any other result that should not be reported
7. Go to Panther Result Tab
8. Filter results by Worklist ID by date and assay
9. “Select All” for Samples to verify

**Note: “Deselect” any Invalid or any samples that you do not want to autoverify.**

1. Verify- tab at bottom of screen
2. Send to LIS- tab at bottom of screen

**Note**: Negative Results will Autoverify in Soft

Positive Results will go to the Instrument Menu in Soft for “Posting”

1. From SoftLab, go to “Interfaces”, and “Instrument Menu”
2. Select “Hologic Panther” (RPANT) for Panther #1 or Hologic Panther 2” (RPAN2) for Panther #2 from Instrument Menu.
3. Select “Loadlist and Today’s Results”, “Not Posted”, “By Sequence”
4. Each order will be highlighted individually. Verify the result against the instrument printout. Click “Post All” for each order to be verified.
5. For any result that is being repeated do not post result.
6. If any Result Comments, ie. Phone reports need to be added:
7. Go to “Lab Result” tab
8. Open “Comment” box and add comment.
9. Save
10. Go back to “Instrument” tab.
11. Post Result
12. Invalid Results that repeat as Invalid due to “volume verification failure sample”.
13. All Invalid results will cross over to Soft as “UNAZ”- “Unable to analyze due to sampling issues caused by specimen integrity. Please resubmit if clinically warranted.
14. “Post” this result when the invalid result is due to error code VVFS- Volume Verification Failure Sample x2.
15. Invalid Results that repeat as Invalid for any other reason.
16. **DO NOT** Post Result (it will say @UNAZ)
17. Go to “Result Entry” and manually enter result.
18. Choose Indeterminate from the keypad and text in an appropriate comment depending on the reason.
19. Cancel any unwanted repeated results from the Instrument Menu

by right clicking, choose Cancel, Save.

1. Save the Instrument Results printout in the HPVG Results Notebook. Write the Tasklist number on the first page.

18. Print a “pending worklist” to check for any outstanding orders.

Resolve all outstanding issues.

**Appendix D**

**HPV/HPVG Sorting, Ordering, Cancelling**

1. **HPV Specimen Sorting**
   1. Retrieve Cytology reports daily from printer in accessioning area:
      1. “Specimens by Retrieval”- these will be older specimens
      2. “Natural Language Search”- these will be specimens aliquotted from the previous one to two days.
      3. If there is more than 1 of each, ie Mondays, sort oldest to newest.

**Note**: Make sure there is a printout for every day even if there are no reports on it.

* + 1. Each report is in LG number order and racks of aliquotted specimens are in LG number order.
  1. “Specimens by Retrieval”
     1. Review report and highlight any orders that specify reflex to 16/18 if positive.
     2. Pull HPV specimens on the list and place in a rack in LG number order going from left to right/ back to front.

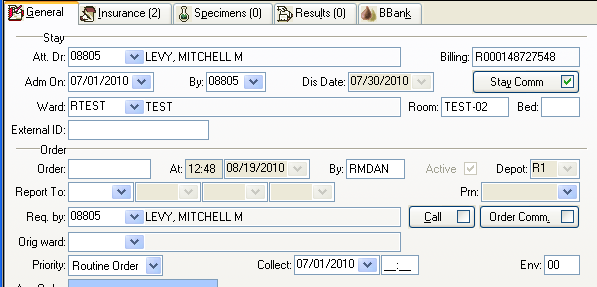
**Note:** Many specimens may have been pulled previously from the Natural Language Search report.

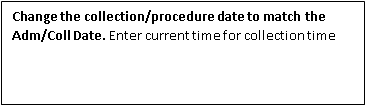
* + - 1. Place a “√” next to orders that were pulled
      2. Place an “X” next to orders with no tube present.
    1. For any specimens reflexed for 16/18:
       1. Highlight the LG number and patient name on the tubes.
  1. “Natural Language Reports”
     1. Review report
        1. Highlight any orders that specify reflex to 16/18 if positive.
        2. Cross out any orders that should not be pulled. Ie:
           1. “Reflex to HR HPV if ASCUS”
           2. “No HPV”
     2. Pull the following specimens from the report:
        1. HPV screen age 30 and over
        2. HPV per physician request
     3. Pull HPV specimens on the list and place in a rack in LG number order from left to right/ back to front.
        1. Place a “√” next to orders that were pulled
     4. For any specimens reflexed for 16/18 highlight the LG number and patient name on the tube.

1. **Ordering HPV1/ HPVG1**
   1. HPV- Soft Code HPV1
      1. Search for patient in Order Entry by MR number
      2. Choose the encounter that matches the PID number on the specimen label.

**Note:** Collection date on tube should match the encounter date. If it does not check with Cytology registration tech.

* + 1. Enter the info on the “General” Tab.





* + 1. Order HPV1
       1. Go to “Specimen Tab and click on “collect/receive”. The collection date/time will default from the previous screen. Click OK.
       2. Go to printer icon, print Standard Label
       3. SCAN the Cytology Case # when the window opens. Do not enter result manually. IF there is no case number associated with the specimen type N/A in the case number space. An example when this might happen: Repeat HPV test without Pap due to QNS.
       4. When it asks if you want to save say” Yes”.
       5. Click OK
       6. Save - Collection Label will print.
       7. Label all specimens so that the LG# is visible but barcode is covered.
  1. Ordering HPV 16,18/45- Soft Code HPVG1
     1. Test requests may come in with the Thin prep vial and ordered at the same time the HPV is ordered under the same case number.
        1. PLEASE REVIEW SHEET THAT COMES WITH THE VIAL TO DETERMINE IF THERE IS A GENOTYPE REQUEST.
     2. Requests may also come in as add-on requests when an HPV test is positive. Add the test on to the same order number as the HPV test. Specimens used for genotype testing will be retrieved by the Molecular Micro techs. Genotyping cannot be performed on a negative HPV test.
     3. Place the ordered tube in the HPVG1 rack to be done.
  2. HPVG Requests
     1. After resulting HPV orders print a “Resulting Worklist” for HPVG1 orders.
     2. Pull Positive HPVs, recap specimen with a foil cap, and place in HPVG1 “To Be Done” rack.
     3. All HPVG1 orders on HPV HR Negative samples will be autocanceled in Soft.