**PROCEDURE: CORO MOLECULAR MICROBIOLOGY COBAS HPV ASSAY**

1. **PRINCIPLE**
   1. Intended Use
      1. **cobas®** HPV for use on the **cobas®** 6800/8800 Systems (**cobas®** HPV) is a qualitative *in vitro* test for the detection of Human Papillomavirus in clinician-collected cervical specimens using an endocervical brush/spatula or broom and placed in the ThinPrep® Pap Test™ PreservCyt® Solution. This test detects the high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.
      2. **cobas®** HPV is indicated for use for routine cervical cancer screening as per professional medical guidelines, including triage of ASC-US cytology, co-testing (or adjunctive screen) with cytology, and HPV primary screening of women to assess the risk for cervical precancer and cancer. Patients should be followed-up in accordance with professional medical guidelines, results from prior screening, medical history, and other risk factors.
      3. HPV-negative cancers of the cervix do occur in rare circumstances. Also, no cancer screening test is 100% sensitive. Use of this device for primary cervical cancer screening should be undertaken after carefully considering the performance characteristics, as well as recommendations of professional guidelines.
   2. Summary and Background
      1. Human papillomavirus (HPV) is a small, non-enveloped, double-stranded DNA virus, with a genome of approximately 8000 nucleotides. There are more than 140 different HPV genotypes and approximately 40 different genotypes can infect the human anogenital mucosa. Fourteen HPV genotypes are classified as carcinogenic or high-risk (HR): 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68. Please note that one of these, HPV66, was recently categorized as “possibly carcinogenic” based on its relatively low prevalence in invasive cervical carcinomas.
      2. Persistent infection with these high-risk HPV genotypes is the central cause of cervical cancer and its precursor cervical intraepithelial neoplasia (CIN). Sexually transmitted infections with HPV are extremely common, with estimates of up to 75% of all women experiencing exposure to HPV at some point. However, most infections clear within 1-2 years. Most cervical cancers and deaths from cervical cancer can be prevented through early detection of pre-cancerous lesions in the cervix, leading to timely treatment. In developed countries with cervical cancer screening programs, the Pap smear has been used since the mid-1950s as the primary tool to detect early precursors to cervical cancer. Although it has decreased the death rates due to cervical cancer dramatically in those countries, the Pap smear and subsequent liquid-based cytology methods require interpretation by highly trained cytopathologists and have a high rate of false negatives. Cytological abnormalities are primarily due to infection with HPV; however, various inflammatory or sampling variations can result in false positive cytology results. Triage of an abnormal cytology result involves repeat testing, colposcopy and biopsy to rule out the presence of high-grade precancerous lesions, (cervical intraepithelial neoplasia of grade 2 or higher; ≥CIN2). Therefore, tests that detect infection with these HR HPV genotypes are now being used increasingly in cervical cancer screening programs to improve the prevention of cervical cancers and clinical patient management. Nucleic acid (DNA) testing by PCR is a non-invasive method for determining the presence of a cervical HPV infection. Proper implementation of nucleic acid testing for HPV may increase the sensitivity of cervical cancer screening programs by detecting high-risk lesions earlier in women 25 years and older and reducing the need for unnecessary colposcopy and treatment in patients 21 and older with atypical squamous cells of undetermined significance (ASC-US) cytology. Therefore, tests that detect infection with these HR HPV genotypes are now being used increasingly in cervical cancer screening programs to improve the prevention of cervical cancers.
      3. The 2006 Consensus Guidelines for the Management of Women with Abnormal Cervical Cancer Screening Tests recognized the utility of using a combination of cervical cytology, tests for HPV detection, and type-specific HPV testing for women undergoing screening for cervical cancer. One of the earliest and most common utilization of HPV testing has been for the management (referral to colposcopy) of women with equivocal cervical cytologic abnormalities (ASC-US). Further, revised and updated guidelines now recommend the combination of cytology and HPV testing (cotesting) as the preferred method of screening in women ≥30 years, with HPV 16/18 genotype-specific testing as an added option to triage women with negative cytology to colposcopy. A later revision provided the option when cotesting to follow up women with low grade squamous intraepithelial lesion (LSIL)/HPV negative results in 12 months rather than refer to colposcopy. Most recently, interim guidance has been issued for HR HPV DNA testing to be used as a first-line primary screening test in women ≥25 years.
      4. Nucleic acid (DNA) testing by PCR is a non-invasive method for determining the presence of a cervical HPV infection. Proper implementation of nucleic acid testing for HPV may increase the sensitivity of cervical cancer screening programs by detecting high-risk lesions earlier in women 25 years and older and reducing the need for unnecessary colposcopy and treatment in patients 21 and older with ASC-US cytology.
      5. **cobas®** HPV is a qualitative real-time PCR test that detects 14 high-risk HPV genotypes. **cobas**® HPV uses primers to define a sequence of approximately 200 nucleotides within the polymorphic L1 region of the HPV genome. A pool of HPV primers present in the Master Mix is designed to amplify HPV DNA from 14 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). **cobas®** HPV utilizes β-globin DNA as an internal control to monitor the entire sample preparation and PCR amplification process so an additional primer pair targets the human β-globin gene (330 base pair amplicon). Fluorescent oligonucleotide probes bind to polymorphic regions within the sequence defined by these primers. In addition, the test utilizes a low titer positive and a negative control.
   3. Sample Preparation
      1. **cobas**® HPV is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas®** 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas**® 6800/8800 software which assigns test results for all tests as positive, negative, or invalid. Results can be reviewed directly on the system screen, exported, or printed as a report.
      2. Nucleic acid (DNA) from patient samples is extracted. In summary, nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors are removed with subsequent wash steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature. External controls (positive and negative) are processed in the same way with each **cobas®** HPV run.
   4. Amplification
      1. A thermostable DNA polymerase enzyme is used for PCR amplification. The HPV and β-globin sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicon from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR master mix, during the first thermal cycling step. However, newly formed amplicon are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.
      2. **cobas**® HPV master mix contains detection probes specific for twelve High Risk HPV target sequences, one detection probe specific for the HPV16 target sequence, one detection probe specific for the HPV18 target sequence and one for β-globin. The amplified signal from twelve high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) is detected using the same fluorescent dye while HPV16, HPV18 and β-globin signals are each detected with their own dedicated fluorescent dye. When not bound to the target sequence, the fluorescent signal of the intact probes is suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the HPV targets and β-globin, respectively.
2. **SPECIMEN REQUIREMENTS**
   1. Specimen Collection
      1. Cervical, vaginal and anal/rectal specimens collected in PreservCyt® Solution have been validated for use with **cobas**® HPV.
      2. Handle all specimens as if they are capable of transmitting infectious agents.
   2. Specimen Transport
      1. Specimens collected in PreservCyt® Solution can be transported at 2-30°C. Transportation of HPV specimens must comply with country, federal, state, and local regulations for the transport of etiologic agents.
   3. Specimen Storage
      1. Specimens collected in PreservCyt® Solution may be stored at 2-30°C for up to 3 months after the date of collection prior to performing **cobas**® HPV. PreservCyt® specimens should not be frozen.
3. **MATERIALS AND REAGENTS**
   1. Reagents
      1. **cobas® HPV** kit includes the following components that are stored at 2-8°C.
         1. Proteinase Solution
         2. Elution Buffer
         3. Master Mix Reagent 1
         4. HPV Master Mix Reagent 2
      2. Reagents not included in the kit and stored at 2-8°C unless stated otherwise:
         1. HPV Positive Control
         2. cobas® Buffer Negative Control
         3. MGP Reagent
         4. Specimen Diluent
         5. Lysis Reagent
         6. Wash Reagent, store at 15-30°C
   2. Materials
      1. cobas omni Processing Plate
      2. cobas omni Amplification Plate
      3. cobas omni Pipette Tips
      4. cobas omni Liquid Waste Container
      5. Solid Waste Container and Bag
   3. Reagent Precautions
      1. Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples, reagents, or controls.
      2. Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
      3. cobas omni Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
      4. Do not allow cobas omni Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
      5. Expended control kits contain pierced vials with residual reagent; special care should be taken during disposal to avoid spills and contact.
      6. cobas® HPV Kit, cobas® HPV Positive Control Kit, cobas® Buffer Negative Control Kit, cobas omni MGP Reagent, and cobas omni Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
      7. Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.
   4. Reagent Storage and Handling
      1. Do not freeze reagents or controls.
      2. Reagents loaded onto the cobas® 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The cobas® 6800/8800 Systems allow reagents to be used only if all of the conditions shown in in the table below are met. The system automatically prevents use of expired reagents. The table below describes the reagent handling conditions enforced by the cobas® 6800/8800 Systems.

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| **Reagent** | **Open-kit stability** | **Number of runs for which this kit can be used** | **On-board stability** |
| **cobas®** HPV | 90 days from first usage | Max 20 runs | Max 20 hours |
| **cobas®** HPV Control Kit | N/A | Max 16 runs | Max 10 hours |
| **cobas®** Buffer Negative Control Kit | N/A | Max 16 runs | Max 10 hours |
| **cobas omni** Lysis Reagent | 30 days from loading\* | N/A | N/A |
| **cobas omni** MGP Reagent | 30 days from loading\* | N/A | N/A |
| **cobas omni** Specimen Diluent | 30 days from loading\* | N/A | N/A |
| **cobas omni** Wash Reagent | 30 days from loading\* | N/A | N/A |

* + 1. Do not use cobas® HPV Kit, cobas® HPV Positive Control Kit, cobas® Buffer Negative Control Kit, or cobas omni reagents after their expiry dates.
    2. Wear laboratory gloves, and laboratory coats when handling samples and reagents. Avoid contaminating gloves when handling samples and controls. Gloves must be changed between handling samples and cobas® HPV Kit, cobas® HPV Positive Control Kit, cobas® Buffer Negative Control Kit and cobas omni reagents to prevent contamination.
    3. Wash hands thoroughly after handling samples and reagents, and after removing the gloves.
    4. Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
    5. If spills occur on the instrument, follow the instructions in the cobas® 6800/8800 Systems User Guide to properly clean and decontaminate the surface of instrument(s).

1. **QUALITY CONTROL**
   1. Quality Control Information for each run:
      1. One cobas® Buffer Negative Control
      2. One HPV Positive Control
      3. Store all controls at 2-8°C.
      4. Controls are stable until the expiration date indicated.
      5. Record QC results on the sheets provided. Include date of testing, kit lot #, control lot #s, expiration dates, and results.
      6. Batch validity is checked within the cobas® 6800 software and is printed with the run report.
      7. The batch is valid if no flags appear for all controls. If the batch is invalid, repeat testing of the entire batch.
      8. Validation of results is performed automatically by the cobas® 6800/8800 software based on negative and positive control performance.
      9. New lot numbers/shipment of HPV kits are QC’d using control kits that have passed QC.
      10. New lot numbers/ shipments of Positive and Negative Control kits are run using HPV kits that have passed QC.
      11. Environmental testing is performed monthly.
   2. Acceptable Limits
      1. For any run, valid results must be obtained for both the Positive and Negative Controls for the cobas® 6800 Software to display the reportable cobas® HPV results from that run.
      2. QC statistics are calculated monthly to define analytic imprecision and to monitor trends over time.
   3. Corrective Actions
      1. The assay will require repeating if either positive or negative controls are not valid.
         1. If the control results are consistently invalid, contact your local Roche Support Network Customer Support Center for technical assistance.
2. **TEST PROCEDURE**
   1. Clean bench tops with 10% bleach followed by 70% alcohol pre and post running the assay.
   2. Collect samples to be tested.
   3. Go to the 6800 **Monitor Tab** and check the taskbar and messages at the top left on the monitor screen.
      1. Address any issues or maintenance due.
   4. Refill reagents and consumables as prompted by the system:
      1. Load wash reagent, lysis reagent and diluent.
      2. Load tip racks, processing plates and amplification plates.
      3. Load Magnetic Glass Particles.
      4. Load test specific reagents.
      5. Load control cassettes.
      6. Replace rack for clotted tips.
   5. Set the system to “Ready”.
      1. In the task overview, ensure that there is no maintenance overdue.
      2. On the Monitoring tab, Choose the Start button.
         1. The system changes to Preparing status.
      3. Wait for the system to change to Ready status before you start loading. This may take 15 minutes.
   6. Organize the HPV runs for the day and make Taskslists.
   7. Load sample racks onto rack trays.
      1. Cobas 6800 has LIS Order Download
      2. For any samples without LIS barcode use Rack Based Ordering, i.e. environmental samples.
         1. Designated HPV racks have Blue labels on them
         2. Sample ID must be entered in Manual Barcode Entry tab.
   8. Load trays with sample racks onto the Sample Supply module and go to the “Batches” tab.
      1. Monitor the “error lane” for any problems.
      2. After the sample barcodes are read it will make the “batch” and list the number of HPVs in the batch.
         1. **Make sure that number matches the expected number of tests**.
         2. Resolve any discrepancies.
   9. Hit the “Start Manually” button to begin processing.
      1. At this point you may go to the Routine Tab> Test Order Status to see the finish time.
   10. Monitor the instrument during processing in the Transfer Module.
       1. Address any errors or issues.
       2. Note: Do not walk away from instrument until samples are moved to the Processing module.
   11. Unload racks and samples when finished pipetting.
       1. Recap tubes with new caps (using p480) and store at room temperature for one week.
   12. Unload consumables at the end of processing:
       1. Remove amplification plates from the analytic module.
       2. Unload empty control cassettes.
       3. Empty solid waste.
       4. Empty liquid waste.
3. **RESULTS INTERPRETATION**
   1. Results
      1. From the **Routine Tab > Control Batch**
      2. Choose the control batch for your run.
      3. From the drop-down list choose Print from both right and left side of screen.
         1. The printout from the left has lot #s of reagents and controls.
         2. The printout from the right has the actual results of controls.
         3. View results
   2. Reporting Results- Refer to Appendix A for LIS resulting instructions.
      1. cobas® HPV automatically detects 14 high risk HPV genotypes (HPV-HR) and/or 12 high risk genotypes with individual typing of HPV16 and HPV18 simultaneously (HPV-GT).
      2. Reported target results for individual samples are valid unless indicated as “Invalid” within the individual target result column.
      3. For invalid target results from PreservCyt® specimens, the original specimen should be re-tested no more than two times to obtain valid results. If the results are still invalid a new specimen should be obtained.
      4. HPV results and interpretation:

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| **Target 1** | **Target 2** | **Target 3** | **Interpretation** |
| Other HR HPV Positive | HPV 16 Positive, HPV 16 Negative, or Invalid | HPV 18 Positive, HPV 18 Negative, or Invalid | Specimen is positive for the DNA of any one of, or combination of the following high risk HPV types: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. |
| Other HR HPV Negative | HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 were undetectable or below the pre-set threshold. |
| Invalid | The result for HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 is invalid. |
| Other HR HPV Positive, Other HR HPV Negative, or Invalid | HPV 16 Positive | HPV 18 Positive, HPV 18 Negative, or Invalid | Specimen is positive for HPV type 16 DNA. |
| HPV 16 Negative | HPV type 16 DNA was undetectable or below the pre-set threshold. |
| Invalid | The result for HPV type 16 is invalid. |
| Other HR HPV Positive, Other HR HPV Negative, or Invalid | HPV 16 Positive, HPV 16 Negative, or Invalid | HPV 18 Positive | Specimen is positive for HPV type 18 DNA. |
| HPV 18 Negative | HPV type 18 DNA was undetectable or below the pre-set threshold. |
| Invalid | The result for HPV type 18 is invalid. |

* + 1. Release Results
       1. Select all test results to be released.
       2. Choose the Release button.
          1. It is possible to release an Invalid result so use caution when releasing.
       3. Test results are sent to the SOFT Instrument Menu for posting.
    2. Invalid Patient Results
       1. An Invalid sample will be retested on the next run. If it repeats as Invalid report as Indeterminate- Suggest collecting new sample.

1. **LIMITATIONS**
   1. Products containing carbomer(s), including vaginal lubricants, creams and gels may interfere with the test and should not be used during or prior to collecting cervical specimens. See package insert for more information.
   2. Use of over-the-counter products Replens™, RepHresh™ Vaginal Gel and RepHresh™ Clean Balance™ Kit has been associated with false-negative results.
   3. Use of Metronidazole Vaginal Gel has been associated with false-negative results.
   4. cobas® HPV detects DNA of the high-risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. This test does not detect DNA of HPV low-risk types (e.g. 6, 11, 42, 43, 44) since there is no clinical utility for testing of low-risk HPV types.
   5. Detection of high-risk HPV 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.
   6. 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.
   7. Infection with HPV is not an indicator of cytologic HSIL or underlying high-grade CIN, nor does it imply that CIN2-3 or cancer will develop. Most women infected with one or more high-risk HPV types do not develop CIN2-3 or cancer.
   8. A negative high-risk HPV result does not exclude the possibility of future cytologic HSIL or underlying CIN2-3 or cancer.
   9. Human β-globin amplification and detection is included in cobas® HPV to differentiate HPV negative specimens from those that do not exhibit HPV signal due to insufficient cell mass in the specimen. All HPV negative specimens must have a valid β-globin signal within a pre-defined range to be identified as valid negatives.
   10. The effects of other potential variables such as vaginal discharge, use of tampons, douching, etc. and specimen collection variables have not been evaluated.
   11. Though rare, mutations within the highly conserved regions of the genomic DNA of Human papillomavirus covered by cobas® HPV’s primers and/or probes may result in failure to detect the presence of the viral DNA.
   12. Results of this test should only be interpreted in conjunction with information available from clinical evaluation of the patient and patient history.
2. **INTERFERENCES**
   1. The effects of endogenous and exogenous substances that may be present in cervical specimens were tested for potential interference. All testing for interference was performed with each potential interfering substance alone as well as with the substance mixed with SiHa (HPV16) and HeLa (HPV18) cell lines at approximately 3 x LoD of cobas® HPV in HPV negative samples.
   2. Endogenous substances tested were cervical mucus, peripheral blood mononuclear cells and whole blood. Exogenous substance testing included 18 over the counter (OTC) feminine hygiene and prescription products that are listed in the package insert. Of OTC feminine hygiene and prescription products tested, Metronidazole Vaginal Gel, Replens™, RepHresh™ Odor Eliminating Vaginal Gel and RepHresh™ Clean Balance™ Feminine Freshness Kit produced false negative results.
   3. Potential interference from the presence of glacial acetic acid was also tested in pools of HPV negative and HPV positive cervical specimens in PreservCyt® Solution. Concentrations up to and including 5% (v/v) of glacial acetic acid were tolerated by the assay.
3. **TECHNICAL ASSISTANCE**
   1. Roche Support Network Customer Support Center at 1-800-526-1247.
4. **REFERENCES**
   1. Roche Cobas HPV Package Insert Rev 1.0
   2. Roche Cobas 6800 Operators Manual