

Laboratory Procedure Manual

Analyte: **High-Risk Human Papillomavirus (HPV)**

Matrix: Self-Collected Vaginal Swabs

Method: Cobas HPV (Roche Diagnostics)

As performed by: Chronic Viral Diseases Branch
Division of High-Consequence Pathogens
and Pathology
National Center for Emerging and Zoonotic
Infectious Diseases

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Important Information for Users

The HPV Laboratory of the Chronic Viral Diseases Branch/CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

Data File Name	Variable Name	SAS Label
HPVSWR_H and HPV_S_H_R	LBXHP2C	Cobas HPV Swab High Risk

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

This protocol describes procedures for DNA extraction and high-risk HPV detection from self-collected vaginal swabs.

DNA extraction is performed with a modified protocol and the commercial QIAamp kit (Qiagen, Valencia, CA). Because of the stability of DNA, extraction of cellular material dried on an inert matrix generally yields DNA of sufficient quality and quantity for PCR testing. The non-invasive subject-performed collection method is generally acceptable to participants. Room-temperature stability simplifies storage and transportation to the laboratory. Typical DNA extraction methods can be used, with modifications to accommodate re-hydration and removal of cellular material from the collection matrix. The protocol was developed for Catch-All™ Sample Collection Swabs (Epicentre, Madison WI) and includes a final concentration and wash of the extract using Ultracell spin columns. Modifications may be required for other types of swabs.

Presence of high-risk HPV in the extracted patient DNA is determined with the Cobas Human Papillomavirus (HPV) Test. This qualitative in-vitro diagnostics test uses oligonucleotide probes labeled with four different fluorescent dyes. The primers target a DNA region of approximately 200 nucleotides within the polymorphic L1 region of the HPV genome it detects 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) in a single analysis. The test reports concurrently the presence of one or more of these types at clinically relevant infection levels, however the test has been modified from the FDA approved format (sample type and extraction) and results cannot be used clinically (See also 12.0 Limitations of the Method).

2. SAFETY PRECAUTIONS:

Appropriate PPE must be worn throughout all lab procedures. General precautions must be followed as outlined in the CCID Safety Manual (CDC). All specimen handling (pre-lysis) is to be performed in a biosafety cabinet.

Cobas buffers HPV MMX, HPV Mg/Mn, (-)C, and HPV(+)C contain sodium azide which may be fatal in contact with skin or if swallowed. Follow procedure as outlined in the OI Safety Manual and Chemical Hygiene Manual.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

Final high-risk HPV results, dates and notes from DNA extraction process are recorded in a password protected database on a secure server. Original data from the Cobas HPV result files are archived. Additional observations and comments kit/reagent lot numbers, and name of operator is recorded on a worksheet accompanying the entire procedure.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES;

CRITERIA FOR SPECIMEN REJECTION

NHANES MEC personnel explain the process for self-collection of vaginal samples to study participants and provide an individually packaged Dacron swab (Catch-All, Epicentre) in a transport sleeve. Vaginal and cervical exfoliated cells are collected by study participants by inserting the swab vaginally (similar to tampon insertion) and rotating as directed. The participants then place the swab in the sleeve and return it to NHANES personnel. The closed sleeve is kept at room temperature and sent to CDC at ambient temperature in weekly or bi-weekly shipment. At the testing laboratory the sample is kept at 4°C until extraction (within 3 weeks).

DNA extracts are stored temporarily at 4 °C or for long term storage, in -80°C freezer.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

N/A

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

6.1 Supplies for Cell Lysis and DNA extraction

6.1.1 Equipment

- Eppendorf Microcentrifuge and rotor for 1.5ml tubes (or similar centrifuge reaching 20,000 x g)
- Biosafety cabinet level II
- Pipettes: 20µl, 200 µl, 1000 µl (Rainin, cat# L-20XLS, L-200XLS, and L-1000XLS)
- Vortex Tube Mixer
- Waterbath, temperature set at 56°C (Daigger, cat# EF8943E) or Beadbath (Lab Armor Beads from Fisher Scientific, cat# 50-550-333)
- Multi-dispense pipettor (Rainin, cat# AR-E1)
- Drummond Pipet-Aid or equivalent

6.1.2 Reagents and Media

Reagents at room temperature:

- Water, nuclease-free (Ambion, Cat. #AM9938)
- Alcohol molecular grade, 200 proof (Sigma, cat# E7023)
- Reagent Alcohol, 70%, in spray bottle (Fisher Scientific, cat# 2546-70-1)
- Physiological Saline (PS) (0.9% Sodium chloride, Fisher Scientific Cat. # LC23485-2 or similar)

- Reagents from QIAamp kit (below): AL, lysis buffer, AW1, wash buffer, AW2, wash buffer, AE, elution buffer
- Tris-HCl/EDTA (TE buffer), DNA suspension buffer, 10mM Tris-HCl, 0.1mM EDTA, (Teknova, Cat. #T0223)
- Sodium Hydroxide, 1.0N solution (Fisher Scientific, cat# AC12426-0010)
- Proteinase K (below)

Reagents at 4°C:

- Dissolved Proteinase K- refer to Reagent Preparation under Procedure

6.1.3 Supplies, Other Materials

- QIAamp Mini Kit (Qiagen, cat.# 51304 for 50 samples or #51306 for 250 samples)
- Amicon Ultra 0.5 Ultracel-100 membrane (Fisher Scientific Cat. No. UFC5100BK)
- Screw-cap polypropylene tubes, 5 ml (TTE Laboratories, Catalog # 580-GRDS)
- Tube racks for 5 ml tubes and 1.5 ml microcentrifuge tubes, heat resistant
- Sterile solution basins (VWR, Cat. No. 21007-972)
- Aerosol barrier pipette tips, 20µl, 200µl, and 1000µl (Glassware, cat. #99810, 91157, and 99930)
- Autorep tips, sterile, 5.0ml and 12.5ml (Rainin, cat. #ENC-5MLS and ENC-12MLS)
- 50ml Falcon tubes (Glassware, cat. #96285)
- Screw-Cap polypropylene storage tubes with O-ring, 1.5ml and 0.5ml (Daigger, cat. #EF4219G and EF4219C)
- 1.5ml tubes (Eppendorf, lock cap, Fisher Scientific, cat. #05-402-25)
- Kaydry towels (Kimberly-Clark, cat. #34721) or similar lab towels
- Biohazard wipers, 4"x 4" (Fisher Scientific, cat. #06-670-35)
- Absorbent bench pads (Fisher Scientific, cat. #15235101)
- Lab coat with ribbed knit cuffs (Daigger, cat. #EF1463)
- Gloves, latex or nitrile, powder-free (Glassware)
- Lab markers, waterproof
- Labels, Extreme temperature tolerant, (Tough-Tags and/or Tough-Spots, Diversified Biotech, cat. #TTLW-1000, T-Spots)
- Autoclavable discard pan

6.1.4 Controls

Nuclease-free water is used as a negative quality control for the DNA extraction procedure as well as PCR assays. The water is processed in the same manner as specimens used in the assay. At least one water controls is included in each DNA extraction. The control and specimens processed are tested by downstream assays.

6.2 Supplies for the Cobas HPV Test

6.2.1 Equipment

- Cobas Z 480 Analyzer (Roche Molecular Diagnostics, cat. #05200881001)
- PCR cabinets
- Pipettes: 20µl, 200 µl, 1000 µl (Rainin, cat# L-20XLS, L-200XLS, and L-1000XLS)
- Vortex Tube Mixer
- PCR Cabinet
- Drummond Pipet-Aid or equivalent

6.2.2 Reagents and Media

Reagents at 2-8°C:

- Cobas 4800 HPV Amplification/Detection Kit, 240 tests/kit, Contents: HPV MMX and HPV Mg/Mn (Roche Molecular Diagnostics, cat. #05238998190)
- Cobas 4800 Control Kit, 10 sets/kit, Contents: HPV (+) C and (-) C (Roche Molecular Diagnostics, cat. #05235855190)

6.2.3 Supplies, Other Materials

- Cobas z 480 Microwell Plate and Sealing Film (Roche Molecular Diagnostics, Cat. #05232724001)
- Tube racks
- Aerosol barrier pipette tips, 20µl, 200µl, and 1000µl
- Kaydry towels (Kimberly-Clark, cat. #34721) or similar lab towels
- Absorbent bench pads (Fisher Scientific, cat. #15235101)
- Lab coat with ribbed knit cuffs (Daigger, cat. #EF1463)
- Gloves, latex or nitrile, powder-free (Glassware)

6.2.4 Quality Control

One set of Cobas 480 HPV Test Positive and Negative Controls are included in each run. For any run; valid results must be obtained for both the Positive and Negative Control for the Cobas 480 software to display the reportable Cobas HPV Test results from that run.

Positive Control – The HPV (+) Control result must be “Valid”. If the HPV(+) Control results are consistently invalid, contact the local Roche office for technical assistance.

Negative control – The (-) Control result must be “Valid. If the (-) Control results are consistently invalid, contact the local Roche office for technical assistance.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

N/A

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

8.1 DNA Extraction

Prepare NHANES – DNA Extraction worksheet with container ID and sample IDs from electronic shipping manifest. Record all reagent and kit lot numbers on sheet, along with the date of extraction. Any unusual observations of the specimens and issues is recorded on the worksheet.

Reagent Preparations:

Prepare Buffer AW1 and Buffer AW2, supplied as a concentrate in the QIAamp kit. Add 125ml 100% Ethanol to Buffer AW1 and 160 ml 100% Ethanol to Buffer AW2 buffers prior to use. Preheat water bath/bead bath to 56°C. For Ultracel preparations, create a 0.1N NaOH solution in 50 ml conical tube by making a 1:10 dilution of 1N NaOH stock solution.

Lysis Process:

1. Turn on biosafety cabinet blower, and clean working surface with 70% ethanol. Cover work surface with absorbent bench pads. Place the following items in the cabinet: a plastic bag-lined discard pan, tubes racks for 5ml tubes, biohazard wipers and self-collected, dry swab samples.
2. On the lab bench covered by an absorbing bench pad, remove from stock containers the total amount of each reagent (PS, buffer AL and Proteinase K) required to make the lysis buffer cocktail for the number of samples being processed and aliquot into a sterile solution basin prior to dispensing.
3. Label one 5 ml polypropylene tube per sample and dispense 460 µl physiological saline, 460 µl buffer AL, and 80 µl Proteinase K into each using multi-dispense pipettor and sterile 12.5ml tips. Alternatively, a master mix can be prepared from these three components prior to dispensation into the specimen tubes. After adding lysis mixture, place caps on the tubes to prevent possible cross-contamination. Transfer rack of prepared 5ml tubes to the biological safety cabinet.

Note: For every container set, process a blank as “negative control” that contains all reagents but no cellular material.

4. Take the cap off the first tube and place upside down on the absorbent pad. Use a biohazard wipe to grasp the handle of the swab from the collection sleeve.

Note: *Newer versions of the Epicentre swab devices have a protective sleeve guard so a wipe may not be needed for grasping the swab handle. However, sleeve guards may not always grasp the swab handle and a wipe will be required for removal of swab from the protective sleeve.*

5. Carefully, without allowing the sponge pad of the swab to touch gloves or any surfaces, insert the self-collected swab into the 5ml tube containing the lysis cocktail so that the sponge pad is completely submerged in the liquid.
6. Using a biohazard wipe to grasp the handle end of the swab, slightly lift the handle and carefully snap off the end of the handle by bending the plastic shaft over the edge of the tube, directed away from your body. The biohazard wipe will block the opening of the tube during this process. The swab should then be short enough to allow the screw cap to fit onto the tube. Discard the snapped-off portion of the swab handle and the biohazard wipe into the discard pan. Recap the tube, and move it to a separate rack for completed samples. Process samples in numerical order.

Note: *In order to avoid cross-contamination, handle only one sample at a time. Change gloves if there is any suspicion that they have been contaminated. Check that all screw caps are tightly closed. Mix each tube by vortexing. Change gloves before removing rack of samples from the cabinet.*

7. Place the rack of tubes in the 56°C waterbath or beadbath and incubate overnight (about 16 h).

Note: *When using a water bath, place weights on tubes if tubes are slightly floating from rack. This will assure that the samples are thoroughly heated. The water level should be below cap level. Do not submerge the tubes underwater.*

QIAamp Extraction Process:

8. For each sample, label a 1.5 ml screw-cap tube for storage and a 1.5 ml microcentrifuge tube for extraction. Using pipettor, add 500 µl 100% ethanol to each 1.5 ml “extraction” tube.
9. After incubation, remove the rack of tubes containing the swabs from the waterbath/beadbath, dry off excess water, and place on a clean absorbent bench pad on the lab bench. Allow the samples to equilibrate to room temperature for at least 15 minutes.

Note: *After overnight lysis, the tubes containing swabs may be handled on lab benchtop.*

10. Vortex each tube for at least 5 seconds. Remove the screw cap from the first sample and discard the cap. Using a 1000 µl pipette, transfer 500 µl of sample to the corresponding 1.5 ml “extraction” microcentrifuge tube. Transfer remaining

sample to the 1.5 ml “storage” screw-cap tube. Close microcentrifuge and storage tubes. Discard the pipette tip and sample tube containing the swab into the autoclave pan. Repeat process for each sample. Change gloves if contamination is suspected. Place “storage” aliquots in -80°C freezer.

11. Label two QIAamp Mini spin columns placed in 2 ml collection tubes for each sample. Vortex each 1.5 ml “extraction” tube thoroughly. Transfer one-half of the DNA-ethanol mixture for each sample into each column using a 1000 µl pipette. Do not moisten the rim of the columns. Close the attached cap and discard pipette tip. Repeat for each sample. Centrifuge in Eppendorf 5415D at 8000 rpm (6000 xg) for 1 min.

Note: Always keep columns in an upright position in rack. All centrifugation steps should be carried out at room temperature (21°C-25°C.)

12. If solution has not entirely passed through the membrane, increase speed and repeat spin.
13. Discard the tubes containing filtrates. Place each spin column in a clean 2ml collection tube.

Note: Wipe off any spillage from the columns before inserting into fresh collection tubes.

14. Carefully, without moistening the rim, add 500 µl buffer AW1 to each column. Replace caps and centrifuge at 8000 rpm (6000 rcf) for 1 min.
15. Discard the collection tubes containing filtrates. Place each spin column in a clean 2 ml collection tube. Carefully, without moistening the rim, add 500 µl buffer AW2 to each column. Replace caps and centrifuge at 13,000 rpm (15,700 xg) for 3 min.
16. Discard the tubes containing filtrates. Place QIAamp spin columns in a new collection tube. Add 100 µl buffer AE directly onto the membrane of each column, cap, and incubate at room temperature for 5 min. Centrifuge at 8000 rpm (6000 xg) for 1 min. Add another 100 µl buffer AE and repeat incubation and centrifugation.

Note: Do not change collection tubes between centrifugations. Filtrates from both centrifugations will be in the same tube. Because sample was divided at beginning of extraction, each sample will have filtrate in two tubes.

Concentration and Purification of the Extracts:

17. Label one set of 0.5ml screw-cap tubes for storage of extracts and set aside. Label one 0.5 Ultracel-100 membrane for each sample.

18. Prepare each 0.5 Ultracel-100 membrane filter by adding 300 µl of 0.1N NaOH into each column. Centrifuge at 14,000 x g for 30 minutes.
19. Remove filtrate from the collection tube and discard filtrate in autoclave pan. Replace the original 0.5 Ultracel-100 membrane filter in the collection tube.
20. Add 300 µl of Nuclease-free water to the membrane filter. Centrifuge columns at 14,000 x g for 30 minutes.
21. Remove filtrate from the collection tube and discard water filtrate in autoclave pan. Replace the original 0.5 Ultracel-100 membrane filter in the collection tube.
22. Place both DNA elution filtrates from the QIAamp collection tubes of the corresponding sample into the Ultracel filter column. Centrifuge at 14,000 x g for 30 min.
23. Remove filtrate from the collection tube and discard filtrate in autoclave pan. Replace the original 0.5 Ultracel-100 membrane filter in the collection tube.
24. Add 300 µl of nuclease free water to the filter. Centrifuge at 14,000 x g for 30 min.
25. Discard filtrate and repeat step 20. Discard collection tube containing the water wash filtrate into an autoclave discard pan.
26. Carefully invert the 0.5 Ultracel-100 membrane filter into a new collection tube. Centrifuge at 1000 x g for five min to collect the retentate (extracted DNA).
27. Bring extracts to 100 µl with TE buffer and transfer the 100 µl into a 0.5 ml O-ring screw capped storage tubes. If not testing immediately, store extract tubes at -20°C.

8.2 High-risk HPV Detection (Cobas HPV)

Test Preparation in Cobas 4800 Software:

1. Create a Work Order file for PCR set-up by preparing the Cobas worksheet, listing sample IDs with barcodes
2. Start the Cobas 4800 Work Order Editor on the Cobas Computer.
3. Enter the user name "CDC" and password "HPV".

4. Select "Create New"
5. Select "PCR only workflow"
6. Select "HPV workflow"
7. Scan microwell plate that will be used in testing in the "Plate ID" section.
8. Click "Next"
9. Choose test subtype "HPV High Risk Panel Plus Genotyping" to report High Risk HPV and separate HPV 16 and HPV 18 results from the drop down menu.
10. Select the test subtype and media type "PreservCyt" from the drop down menu.
11. Select "C1" on the MWPPosition drop down menu.
12. Click on the Mode drop-down arrow. Select "Create" mode.
13. Click on the Barcode field to highlight and scan each sample barcode from the Cobas worksheet. The sample information displays in the Samples pane on the right.
14. Click on the Mode drop-down arrow. Select "Edit" mode.
15. Click on "INSERTBARCODE N/A PosCtrl A1" in the Samples field.
16. Click on Barcode field to highlight the field in the Attributes box.
17. Scan the Positive Control vial "Cobas HPV (+) C"
18. Edit the last 4 digits of the Cobas HPV (+) Control. (i.e. January 21st, 1st run of day=A211)
 - The first digit corresponding to Month of the run date.

Month	Letter
January	A
February	B
March	C
April	D
May	E
June	F
July	G
August	H
September	I
October	J
November	K
December	L

- The second and third digits correspond to the day of the run.
- The fourth digit corresponds to the run ID.

19. Click on the Mode drop-down arrow. Select “Edit” mode.
20. Click on “INSERTBARCODE N/A NegCtrl B1” in the Samples field.
21. Click on Barcode field to highlight the field in the Attributes box.
22. Scan the Negative Control vial “Cobas HPV (-) C”
Repeat steps 18 a-c for editing the last four digits of the NegControl.
23. Click “Save” to open the Save As dialog box.
24. Enter a file name (NH_run ID number) and click “Save”

HPV Detection by PCR – Setup:

Note: *One set of Cobas 4800 HPV Test Positive and Negative Controls are included in each run. The controls are liquid and ready-to-use.*

- Open the Cobas 4800 program, Click on “New Run”
- Select “HPV PCR Only Workflow” on the Test Type field.
- Enter Run ID in the “Run Name” field (Same as file name NH_run ID number)
- Click “OK”

25. Prepare the Cobas 4800 HPV working master mix in PCR Reagent Only BSC:

For a run of up to 24 tests, add 240 µL of HPV Mg/Mn to one vial of HPV MMX (0.5mL from 240 Test Kit).

Thoroughly mix working master mix by carefully inverting the vial(s). Do not vortex the working master mix.

Note: *The PCR-only run must be started within 90 minutes of addition of HPV Mg/Mn to the HPV MMX. The system does not monitor the length of time after addition of the prepared samples to working master mix in the PCR only workflow. The end user must ensure that amplification and detection is started within the allotted time.*

26. In PCR Template Only BSC, transfer 25 µL of working master mix to each of the required wells in the microwell plate.

27. Add DNA from test samples

DNA extracted from dry swabs as detailed above are to be added in a 1: 25 dilution: Manually transfer 5 uL of DNA extract to 120 uL of 0.1M -TE Buffer. Mix thoroughly and add 25 uL of this dilution to the corresponding well in the microwell plate.

28. Seal the microwell plate. Centrifuge the microwell plate using a swinging bucket rotor for at least 5 seconds at 1500 rcf.

29. Transport the plate to the Cobas z 480 analyzer and start the amplification and detection run.
30. Press the load button on the Cobas z 480 analyzer.
31. Load Microwell Plate. Press the load button again to close.
32. Click on "Browse" to select the file name on the Cobas 4800 program.
 - When the amplification and detection run is completed, click 'Show Results.' Review and accept results in Results work area (the accepted results are indicated by a checkmark).
 - Select results and click on the print button for results report.
 - Select results and click 'Export' to export and save results in XML format on a USB thumb drive.
 - Transfer the complete result table and paste into the project Access table "Cobas Results".
33. Remove the microwell plate from the extended plate loader and discard.

9. REPORTABLE RANGE OF RESULTS

The Cobas HPV test is qualitative and only determines the presence or absence of high-risk HPV. If any analyte HPV 16, HPV18 or Other High-risk is indicated as positive (POS) in the Cobas result file the result for the sample ID will be recorded as positive. If all of the HPV analytes are negative (NEG) in the Cobas result file the result for the sample ID will be recorded as negative. If any of the analytes are indicated as invalid in the Cobas result file, the DNA from that sample will be re-tested one time to obtain valid results. If the repeated result are still invalid the final result will be recorded as invalid.

10. QUALITY CONTROL (QC) PROCEDURES

The HPV (+) Control result must be 'Valid.' The (-) Control result must be 'Valid' and negative for Other HPV, HPV16 and HPV18. These QC measure are interpreted by the Cobas software and result in an invalid run if any fails.

The negative extraction control - "blank" (see under 8.1 step 3) must be "INVALID" Once results are interpreted and recorded successfully, the PCR plate containing amplified product can be discarded in biological waste bin.

10% of all Cobas tests are repeated within 3 months to assess and monitor reproducibility.

All observations and comments including date of testing, kit/reagent lot numbers, and name of operator should be recorded in the Cobas worksheet.

The technical supervisor reviews the results and signs the print copy of the Cobas test report.

Any incidences or problems are documented on the worksheet and reported to the supervisor.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

The HPV (+) Control result must be 'Valid.' The (-) Control result must be 'Valid.'
If a sample tests as Invalid by the Cobas HPV test, the DNA from that sample is re-tested one time to obtain valid results. If the repeated result are still invalid the final result will be recorded as invalid.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

- Detection of HPV is dependent on the number of viral genomes present in the sample and may be affected by sample collection methods, patient factors, stage of infection and the presence of interfering substances, particularly in self-collected samples.
- The Cobas HPV test 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.
- The performance of the Cobas HPV test has not been adequately established for HPV vaccinated individuals.
- The presence of PCR inhibitors may cause false negative or invalid results.
- Certain body fluids or other specimen contaminants have been shown to inhibit PCR amplification and may give false negative or invalid results.
- A negative result does not preclude the presence of types not included in the probe set.
- The performance characteristics of the Cobas HPV Test have not been validated in connection with a self-collected vaginal swab and manual DNA extraction. Therefore the results of this test should not be used for the diagnosis, treatment, or assessment of patient health and management.

13. REFERENCE RANGES (NORMAL VALUES)

N/A

14. CRITICAL CALL RESULTS ("PANIC VALUES")

N/A

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

All specimens are stored in 4°C conditions up to two weeks during processing and testing unless specified differently by the procedure.

16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

No alternative test method is available. In the event that the DNA extraction or the Cobas HPV test fails specimens are stored at 4°C if the procedure can be repeated within 2 weeks or at -80°C if longer storage is required.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

HPV genotyping results are submitted to Westat electronically within 21 days of receipt of the specimen. Result files in the format of the NHANES shipping manifest are uploaded to the Westat ftp server. Unexpected delays are communicated to Westat.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Original biological specimens are collected at the NHANES Mobile Examination Clinics and shipped to the HPV laboratory at CDC via FedEx. At the HPV lab, all specimens and resulting DNA extracts are tracked via a LIMS system.

19. Summary Statistics and QC graphs

N/A

REFERENCES

QIAamp DNA Mini Kit Handbook, Version Date February 2003. QIAGEN Corp.

Cobas 4800 system Operator's Manual Software Version 1.0, 2009, Roche
Diagnostics Ltd