Laboratory Procedure Manual

Analyte: **Human Papillomavirus (HPV)**

Matrix: **DNA Extraction from Self-collected Vaginal Swabs**

Method: **Catch-All Sample Collection Swabs**

Method No.:

Revised:

as performed by: *Viral Exanthems and Herpes Virus Branch*
Division of Viral and Rickettsial Diseases
National Center for Infectious Diseases

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Important Information for Users

The National Center for Infectious Diseases/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.

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. Typical DNA extraction methods can be used, with modifications to accommodate re-hydration and removal of cellular material from the collection matrix.

The cellular material extracted from the vaginal swab was used for the Roche Reverse Line-Blot HPV Typing Assay (Prototype) and Digene Hybrid Capture II testing.

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

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, Cat. No. QFC091H). Modifications may be required for other types of swabs.

2. SAFETY PRECAUTIONS

HANDLE ALL ASSAY SPECIMENS AND DISPOSED MATERIALS AS IF CAPABLE OF TRANSMITTING INFECTIOUS AGENTS.

A. Patient specimens should be handled at the BSL 2 safety level as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 1984, pages 12–16.

B. Do not pipette by mouth.

C. Wear disposable powder-free gloves while handling reagents or specimens. Wash hands thoroughly after performing the test.

D. All materials used in this assay, including reagents and specimens, should be disposed of in a manner that will inactivate infectious agents.

(1) Solid Wastes: Autoclave.

(2) Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0% (1:5 dilution of household bleach). Allow 30 minutes for decontamination before disposal.

(3) SPILLS: Non-base-containing spills should be wiped thoroughly with a 5% sodium hypochlorite solution (full-strength household bleach). Base-containing spills should be neutralized, wiped dry, and then the spill areas should be wiped with a 5% sodium hypochlorite solution. The wiped area should be covered with absorbent material, saturated with a 5% sodium hypochlorite solution and allowed to stand for at least 10 minutes. A glass or plastic cover or tray can be used to reduce exposure to fumes. All wiping materials should be treated as hazardous waste.

E. Handling Precautions

Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not failing within the established time and temperature ranges must be repeated.

(1) Do not use the reagents beyond the expiration date on the outer box label.

(2) The PCR Test Procedure, Quality Control and the Interpretation of Specimen Results must be followed closely to obtain reliable test results.

(3) It is important to pipette the exact reagent volume indicated and to mix well after each reagent addition. Failure to do so could result in erroneous test results. Ensuring that the noted color changes occur will help confirm that these conditions have been met.

(4) These components have been tested as a unit. Do not interchange components from other sources or from different lots.

(5) Care should be taken to deliver the correct volumes of reagents to the reaction tubes and

2. Equipment and Supplies

A. Qiagen QIAmp Mini Kit (Catalog 51304 for 50 samples or 51306 for 250 samples)

B. Microcon YM-100 Columns (Fisher Scientific Cat. No. 42414)
C. 12x75 mm capped polypropylene tubes (Falcon, Catalog # 352063)
D. Microcentrifuge tubes, 1.5ml polypropylene
E. Tube racks for 12x75 tubes and 1.5 ml microcentrifuge tubes, heat resistant
F. Hood, Biosafety level II
G. Autoclavable discard pan
H. Pipettors and aerosol-barrier tips for volumes 50–1000 µL
I. Vortex tube mixer
J. Lab coat with ribbed knit cuffs
K. Gloves, latex or nitrile, powder-free
L. Eppendorf Microcentrifuge, with rotor for 1.5-ml tubes (or similar centrifuge capable of 20,000 × *g*)
M. Water bath for 56°C
N. Lab markers, waterproof
O. Waterproof labels (such as Microtube Tough-Tags, Diversified Biotech Catalog No. TTLW-1000)
P. KayDry towels (Kimberly-Clark Catalog No. 34721) or similar lab towels
Q. Absorbent lab bench covering
R. Scissors
S. Lab markers

3. Reagents (Room Temperature Storage)

A. Water, distilled, deionized
B. Ethanol, 100%
C. Ethanol, 70%
D. Physiologic saline (PS) (0.9% Sodium chloride, Fisher Scientific Catalog No. LC23485-2 or similar)
E. Buffer AL (from QIAmp kit)
F. Buffer AW1 (from QIAmp kit)
G. Buffer AW2 (from QIAmp kit)
H. Buffer AE (from QIAmp kit)

4. Reagents (4°C Storage)

Proteinase K (from QIAmp kit)

5. Procedure Operating Instructions

Notes: Wear lab coat and gloves. Preheat water bath to 56°C. Prior to beginning protocol, turn on biosafety cabinet blower, and clean working surface with 70% ethanol. Cover work surface with absorbent pads. Place the following items in the cabinet: a plastic bag-lined discard pan, pipettors and tips, PS, buffer AL and Proteinase K from QIAmp kit, labeled 12x75 tubes in rack (in numerical order: include a “contamination

control" tube after every 20 samples – label these A, B, C,….), swabs in rack (also in numerical order), at least one labeled tube for a water blank (negative control), precut 70mm x 70mm KayDry squares (one per sample), vortex mixer, gloves, empty 12 x 75mm tube rack.

The first two protocol steps should be done in a biological safety cabinet. All centrifugation steps should be carried out at room temperature.

(1) To each 12x75 mm polypropylene tube, add 460 µL of PS, 460 µL of buffer AL, and 80 µL of proteinase K. (This is more than twice the amount of lysis buffer cocktail recommended in the QIAmp protocol, but the extra volume insures that the swabs are completely covered, and the lysis is complete.) Recap all tubes.

(2) In order to avoid cross contamination, handle only one sample at a time. Take the cap off the first tube and place upside down on the absorbent pad. Take the top of the plastic sleeve off of the corresponding swab container and discard in pan. Use the KayDry square to grasp the handle end of the swab and transfer it to into the opened polypropylene tube, making sure that the sponge pad of the swab is not touched. Insert the swab in the tube so that the sponge pad is completely submerged in the liquid. With the KayDry square still on the handle end, 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 KayDry square will block the opening of the tube during this process. The swab should then be short enough to allow the snap cap to fit on the tube. Discard the snapped-off portion of the swab handle and the KayDry square in the discard pan. Recap the tube, and move it to a separate rack for completed samples. Process samples in numerical order. Change gloves every 20 samples, or if there is any suspicion that they have been contaminated. For every 20 samples, process a blank "contamination control" that contains all reagents but no cellular material.

    Check that all snap caps are tightly closed. Mix each tube by vortexing. Change gloves before removing rack of samples from the cabinet.

(3) Place the rack of tubes in the 56°C waterbath and incubate overnight or at least 12 hours. Note: Place weights on tubes or use cap-locks so caps do not pop open.

(4) Label two sets of 1.5 mL microcentrifuge tubes. Add 500 µL of 100% ethanol using pipettor to set of tubes for extraction. Place racked and numerically ordered microcentrifuge tubes under the hood. Move samples from the waterbath to the hood. Make sure snap caps are tight, and mix tubes by vortexing. Remove the snap cap from the first sample and discard the cap in the autoclave pan. Using 1000 µL pipettor transfer a 500 µL sample to the first microcentrifuge tube (extraction) and the remainder to the second (storage). Discard the pipette tip and sample tube containing swab into the autoclave pan. Repeat process for each sample. Change gloves if contamination is noted. Place storage aliquots in –20°C freezer.

(5) Move tubes for extraction to benchtop.

(6) Label two QIAmp Mini columns in 2-ml collection tubes for each sample. Transfer one-half of the DNA-ethanol mixture for each sample onto each column using 1000 µL pipettor. (Procedure follows QIAmp protocol from this point on.) Do not moisten the rim of the columns. Discard pipette tip and cap tubes. Repeat for each sample. Centrifuge at 6000 × *g* for one minute. Note: Always keep columns in an upright position in rack.

(7) If solution has not entirely passed through the membrane, increase speed and repeat spin.

(8) Place each column in a clean collection tube. Discard the tubes containing filtrates. Note: Wipe off any spillage from the columns before inserting into fresh collection tubes.

(9) Carefully, without moistening the rim, add 600 µL of buffer AW1 to each column. Replace caps and centrifuge at 6000 × *g* for 1 minute.

(10) Place each column in a clean collection tube. Discard the tubes containing filtrates. Note: Wipe off any spillage from the columns before inserting into fresh collection tubes.

(11) Carefully, without moistening the rim, add 600 µL of buffer AW2 to each column. Replace caps and centrifuge at maximum speed for 3 minutes.

(12) Place each column in a clean collection tube. Discard the tubes containing filtrates. Note: Wipe off any spillage from the columns before inserting into fresh collection tubes.

(13) Add 100 µL of buffer AE (at room temp.) directly onto the membrane of each column, cap, and incubate at room temp. for 5 minutes. Centrifuge at 6000 × *g* for 1 minute. Do not discard filtrate. Do not change collection tubes.

(14) Add 100 µL of buffer AE (at room temp.) directly onto the membrane of each column, cap, and incubate at room temperature for 5 minutes. Centrifuge at 6000 × *g* for 1 minute. Do not discard filtrate. Do not change collection tubes.

(15) Place combined filtrates for each sample into a Microcon 100 (Fisher Scientific). Wash 2× with deionized water. Bring retentates to 100 µL with water. If not testing immediately, store DNA extracts at –20 OC.

(16) For Hybrid Capture II, add 50 µL of extracted sample to 200 µL of PBS. Add 125 µL of Digene denaturation solution to each tube and incubate at 65°C for 45 min. Refrigerate samples overnight, then proceed with Hybrid Capture II protocol. For PGMY primer amplification, use 5 µL from remaining extracted sample.