Chapter 4 - Collection and Handling of Specimens for HPV Testing

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Contents

4.1 INTRODUCTION

The biologic specimen collected for human papillomavirus (HPV) testing is the first step towards providing reliable data on HPV to address important basic science, epidemiologic, and clinical questions about HPV and the impact of HPV vaccination on various biologic endpoints. The specimen provides the window to these processes, and the best laboratory assays cannot overcome specimen quality limitations. The importance of this step can be overlooked. As the laboratory is not usually responsible for actually collecting the specimen, laboratories need to provide advice about recommended methods to those collecting and submitting specimens. In addition, laboratories should provide (or review/approve) clear directions and SOPs for specimen collection, storage, and transmission to the laboratory. While not discussed in detail in this manual, laboratories need to establish a specimen labelling system so that individual specimens can be tracked through receipt, processing, testing and storage. Identifiers should be unique alphanumeric identification (ID). Labels for storage are ideally printed on freezer resistant labels. Incorporating bar code identification of specimens and purchase of barcode readers is an investment that will facilitate speed and accuracy of specimen handling.

As HPV is cell-associated, it should be kept in mind that (with the exception of cell free assays occasionally used in cancer monitoring) results of HPV detection assays (i.e., HPV deoxyribonucleic or ribonucleic acid (DNA or RNA)) only reflect HPV from the anatomic sites sampled. A consistent method of specimen collection and handling is needed to provide reliable clinical results, and thus regulatory body-approved clinical assays are restricted to specific specimen types, collection methods and collection media. In surveillance and research settings specimen standardization is also essential to yield comparable results over time and between studies; however, there is generally more flexibility in establishing a sampling method. Different methods of specimen collection and handling, even from the same anatomic site, can influence the final result, as can method of extraction and volume of extracts used in each assay. A variety of specimen collection and handling methods will provide satisfactory results. Methods of sampling vary in their cost and ease of implementation. Specimen processing and nucleic acid extraction must be matched to the collection method and media used. Commercial/clinical kits for HPV testing may specify collection method and processing. In this situation the manufacturer's recommendation must be followed unless an alternative method has been validated and confirmed to provide satisfactory results.

Residual clinical specimens collected for other purposes (e.g., formalin-fixed paraffin-embedded tissues of cancer lesions or residual swabs for detection of chlamydia and gonorrhea) may be a convenient source for HPV surveillance and research. This requires validation of the assay with the residual sample and verification that processes for collection, testing and retrieving residual specimens will prevent cross-contamination.

This chapter will provide information and one example protocol for the most frequently used sampling methods. However, some methods reported in the literature that have specific research or epidemiologic uses are not covered, such as plucked hairs, emery paper exfoliation, swabbing of different anatomic sites, and unfixed tissue specimens.

4.2 QUALITY

Specimen collection, labeling, storage, and processing fall in the preanalytical phase of laboratory quality management as described in Chapter 3 Laboratory Quality Assurance. To re-emphasize briefly, for clinical applications and for each surveillance or research study, it is essential to use consistent, validated methods for each step of the process. This includes collection device, process for specimen collection (i.e., how and where device is applied), collection media, and storage conditions. For commercial assays, manufacturer's instructions need to be followed. For any changes in the process, the laboratory must validate that the specimens yield comparable results. Finally, for applications that combine clinical and surveillance or research testing, the clinical specimen should be collected first.

4.3 SAFETY

Specimens should be treated as biohazardous material and should be handled under Biologic Safety Level 2 conditions unless co-infection with more hazardous microorganisms warrants a higher BSL. All laboratory work with specimens should be conducted in a biosafety cabinet. Gloves, laboratory coats with tight-fitting cuffs, and protective eyewear should be worn during specimen processing. Methanol and ethanol, which are components in some collection media, are flammable, reactive with sodium hypochlorite (bleach), and require special handling and storage. Each laboratory and their personnel should follow safety requirements of their respective institutions, but readers are encouraged to refer to most recent World Health Organization (WHO) laboratory biosafety manual for more information.[1]

4.4 CERVICAL EXFOLIATED CELL SPECIMENS (HEALTH CARE PROVIDER COLLECTED)

The goal is to provide a cellular specimen with preserved DNA from the site of infection. Collection devices and methods used for obtaining specimens for routine cervical cytology generally yield an appropriate specimen for HPV testing. The cervical transformation zone is the site of origin of most cervical neoplastic lesions, and, as in sampling for cervical cytology, should be targeted for specimen collection. A separate endocervical specimen may be desirable. This requires a health-care professional trained in performing pelvic examination, examination table, and private examination room. A variety of collection devices can be used, e.g., broom, brush, swab, spatula. The swab material also varies, Dacron (DuPont, Wilmington, Delaware, US), cotton, sponge, etc. These are designed for single use and are generally packaged individually and opened just prior to use by the operator using gloved hands. In most applications, the collection device is either placed in media or rinsed in media to release cells. The specimen container should be labeled prior to the start of sample collection to avoid confusion. The protocol below (Equipment and supplies, Procedure) is one example.

4.4.1 Equipment and supplies

- Refrigerator (4°C, range 2°C to 8°C)
- Pelvic examination table
- Gown, sheet for participant
- Examination light
- Sterile vaginal speculum
- Gloves
- Cervical brush/broom (device matched to collection media)
- Dacron swabs
- Specimen vials with collection media
- Specimen labels
- Specimen rack or device to hold tube upright after collection
- Biohazard waste container

4.4.2 Procedure

1. Assemble all materials required to collect specimen. Check the expiration date on collection media or kit to verify it has not expired. [Samples collected using an expired media will need to be re-collected to ensure accurate results]. Label collection vial with identifiers as appropriate (name, subject ID, date of birth, date of collection). For research applications, study ID replaces name, and other information may be added as required by study/surveillance protocol.

2. Explain procedure to the participant and verify their identity. Allow her to disrobe in privacy and arrange sheet for modesty. Two persons should be present during the examination. Position patient for pelvic examination and place speculum to allow visualization of cervix.

3. Remove excess cervical mucous with swab and discard swab into biohazard waste container. Collect cellular specimen. For cytobroom, insert central bristles into endocervix so that outer bristles lie on ectocervix, and rotate device counterclockwise full circle three times. Remove the cytobroom from the canal avoiding any contact of the brush with any other object. **Manufacturers of most collection devices provide directions and illustrations demonstrating a recommended technique for specimen collection. This may be substituted for the general guide presented. It is important to use a consistent method.**

4. Place collection device in vial with specimen collection media. Collection device is either left in media, or cells are dislodged, and device is discarded. This depends on the collection media. Manufacturer's guidelines must be followed as to appropriate collection device and method of cell release.

    - For aqueous media l such as Digene Specimen Transport Medium (STM; Qiagen, Germantown, MD): snap or cut the handle of the collection device so that the swab or brush remains in the media and the lid on the vial may be snapped shut or tightly closed. The bristles should be covered with liquid.

- For PreservCyt medium (Hologic): vigorously press the cytobroom against the bottom of the vial at least five times, splaying the bristles and twisting to release all cellular material. Remove cytobroom and discard into biohazard waste. Tighten screw-cap on collection vial, verifying closure lines are in position indicating tight seal.
- For SurePath medium (Becton Dickinson [BD], Franklin Lakes, NJ, USA): detach the bristle/broom end of the collection device and leave it in the collection vial. Discard the handle into biohazard waste. Tighten the lid on the vial.

NOTE: not all collection media options used in research/surveillance are described, for example – Aptima Multitest Collection Medium (Hologic, Marlborough, MA, USA), designed for detection of non-HPV sexually transmitted infections, lyses cells and can be used in HPV assays, when properly validated.

5. Allow the participant to dress in private and clean examination table and work area.

6. Store specimens under conditions appropriate for the collection media, and ship under conditions maintaining storage temperature. For example:

    - Digene STM: can be kept at room temperature up to 14 days. Stable at 4°C for three weeks. For longer storage, freeze at -20°C (range -15°C to -32°C).
    - PreservCyt: Can be kept at room temperature up to 14 days. Stable at 4°C for at least three months. Freezing is not recommended, although cells may be pelleted and stored at -20°C.
    - SurePath: Can be kept at room temperature up to 14 days. Stable at 4°C for at least three months. Freezing is not recommended.

7. Make sure that the laboratory requisition form accompanies the sample (confirmation of the same patient information). For surveillance or research, the specimen transport log replaces the requisition form.

4.5 SELF-COLLECTED CERVICOVAGINAL SWAB SPECIMENS

Self-collected specimens allow patients to participate in cervical cancer screening without undergoing the intimate and uncomfortable, and in some settings, culturally unacceptable process, of a pelvic examination. In addition, it makes cervical cancer screening accessible in areas that lack personnel trained to conduct a speculum examination and reduces the barriers to participating in research/surveillance studies. Several studies have demonstrated comparable results for HPV detection between self-collected and clinician collected specimens[2-4] and this forms part of the WHO Guideline for Screening and Treatment of Cervical Pre-cancer Lesions for Cervical Cancer Prevention (2021). While the pelvic examination provides additional important information about women's health as well as visual clues about the status of cervical disease, providing a self-collection option can achieve the essential goal of increasing participation in cervical cancer screening. HPV testing on self-collected cervicovaginal specimens has been widely implemented in many countries as a cervical screening method, including mailing kits for at-home collection or the use of self-sampling when performed in a healthcare setting, which could include traditional clinics as well as other locations such as pharmacies or mobile clinics.

Matching the collection device, media, and method to the intended HPV assay is essential, as the cervical-vaginal collection may not include as many cells from the site of the lesion as that collected from the cervix after visualization. As a result, amplification-based (PCR) HPV tests are recommended for HPV testing of self-collected samples. A variety of collection devices have been used. The simplest, used in some surveillance studies being a swab that is placed directly in plastic sleeve (dry) for shipment. Other devices include Evalyn Brush (Rovers Medical Devices, Oss, The Netherlands), Copan FLOQswab (Copan, Brescia, Italy), Qvintip device (Aprovix, Solna, Sweden), and Vibra-Brush (Rovers Medical Devices).

For surveillance, as in the example below, Aptima Multitest Swab and collection media have been used. The approaches vary in cost and extent of validation with HPV assays.

Whether collection is performed at home or in a healthcare setting, clear instructions must be provided to women to assure that the collection is standardized and adequate to represent the cervical-vaginal pool of exfoliated cells. Instructions should specify how to insert the device into the vagina, depth of insertion, and mode of collection (i.e., number of turns), as well as need to avoid touching the swab or brush end of the device. Instructions should be given verbally, written, and with diagrams. In a health care or research setting, a private area with hand washing facilities is required. An example protocol for surveillance study (Equipment and supplies, Procedure) follows:

4.5.1 Equipment and supplies

- Refrigerator (4°C, range 2°C to 8°C)
- Aptima Multitest Swab specimen collection kit
- Specimen labels
- Specimen rack or device to hold tube upright after collection
- Gloves, laboratory coats, protective eyewear for health-care professional
- Soap, warm water, and paper towels
- Written instructions, with visual aids for key steps

4.5.2 Procedure

1. Study professional should assemble all materials required to collect specimen. They should label collection tube with subject ID, date and other information required by study/surveillance protocol. Study personnel should explain the vaginal swab collection technique to study participant, verify their identity and then provide the collection kit.

2. PARTICIPANT INSTRUCTIONS: Wash and dry hands before beginning.

    a. Unscrew the cap of the short tube with liquid and place it on the clean surface. Remove the swab from the package. Do not touch the soft tip of the swab and do not let swab tip touch anything.

    b. Hold the swab shaft with thumb and forefinger and carefully insert the swab deeply into vagina, gently advance until meeting resistance (similar to inserting a tampon).

c. Gently rotate the swab for 10-30 seconds and make sure the swab touches the walls of the vagina.

d. Carefully place the swab into a short tube with liquid, break the swab shaft at the score line against the side of the tube, and close top of the tube tightly.

e. Place the collection tube in the designated area in the bathroom and wash hands with soap and water.

f. Return collection tube to study personnel. Notify study personnel if any problems occurred during collection (e.g. dropped swab, touched swab, spilled media) as a new collection may be needed.

3. Study personnel should make sure that the top is tightly closed to prevent leakage during shipment.

4. Store specimens at refrigerator (2°C to 8°C) and ship to laboratory for HPV testing. The Aptima collection kit, specimens may be stored at refrigerator for up to 60 days before testing or at -15°C to -82°C for long term storage.

4.6 URINE SPECIMENS – FEMALES

Urine is a noninvasive specimen that is easy to collect, although careful methods are needed for HPV testing. While urinalysis assays are based on 'clean catch' urine specimens in which labia are cleaned and urine is collected midstream, avoiding cervical-vaginal cells, urine for HPV testing needs to be optimized to maximize the cervical-vaginal component. First-void urine, which is the initial 20-30 mL of urine flow, should be used for HPV testing as it collects most of the debris from cervical exfoliated cells and contains higher amount of human and HPV DNA compared to random or mid-stream urine.[5]

Urine specimens have been used in research and surveillance studies. Similar to self-collected cervical-vaginal swabs, self-collected urine specimens have the potential to overcome barriers to participation in cervical screening. While studies have shown that HPV detection in urine specimens can be comparable to results from self-collected cervicovaginal and clinician collected cervical specimens[6, 7], adjustment to assay conditions and concentration methods are generally required. As noted earlier, first-void urine gives optimal results and while this can be achieved with a standard urine cup, a special collection device, Colli-Pee (Novosanis, Wijnegem, Belgium) has been developed to simplify collection of a first-void specimen. The Colli-Pee device can be pre-filled with various preservative media to increase the stability of urine specimens.

Due to anatomical differences, urine is poorly representative of male HPV genitals, and this method is generally restricted to females. Clear instructions must be provided to women to assure that the collection is standardized. Study participants should not urinate two hours before collecting specimens and need to refrain from labial washing or vaginal douching prior to the study visit. Instructions should specify how to use the device or cup, emphasizing the need to collect the first void of urine. A private area with toilet and hand washing facilities is required. Spare labels should be printed in case they are needed. An example protocol for surveillance study (Equipment and supplies, Procedure) follows:

4.6.1 Equipment and supplies

- Refrigerator (4°C, range 2°C to 8°C)
- Colli-Pee device prefilled with preservative media (Colli-Pee UCM FV-5020; Novosanis, Wijnegem, Belgium)
- Specimen labels
- Specimen racks or devices to hold tube upright before and after collection
- Gloves, laboratory coats, protective eyewear
- Biohazard waste container
- Soap, warm water, and paper towels
- Written instructions with visual aids for critical steps

4.6.2 Procedure

1. Colli-Pee urine collection kit should be assembled – The lid of the collection tube with preservative removed, and labeled tube screwed into the device. Study personnel should verify participant's identity and study ID, explain the urine collection procedure to participant, and then provide the collection kit. Ask the participant to return the urine-filled Colli-Pee assembled as given.

2. Study participants should be given written instructions with visual aids for key steps, the collection kit, and shown into the private toilet area.

3. PARTICIPANT INSTRUCTIONS:

    a. Wash hands with soap and water before collection. Do not wash or clean the genital area before urine collection.

    b. Hold the collection tube with Colli-Pee housing and urinate through the housing part. There is no need to interrupt the urine stream as collection device will automatically collect the correct volume of first-void urine. Excess urine will pass through other end of the Colli-Pee housing into the toilet.

    c. Notify study personnel of any problems during collection, such as spilling, missing first void, touching surface. Recollection may be needed.

    d. Return the still-assembled Colli-Pee device to the study personnel and wash your hands with soap and water.

4. Carefully disconnect the Colli-Pee housing from the collection tube and then close the cap of the tube tightly until it makes a click sound. Dispose the Colli-Pee housing device in a designated waste container.

5. Specimens can be stored at room temperature for 7 days or refrigerated (2°C to 8°C) for 14 days before HPV testing. Ship to laboratory for HPV testing maintaining the storage temperature. Store at -80°C (range -56°C to -82°C) for long-term storage.

6. If recollection is indicated due to a problem during collection, discuss a return visit with the participant according to a pre-determined study protocol.

4.7 MALE EXTERNAL GENITAL SPECIMENS

HPV infection in men causes genital warts, penile and anal cancers. Fewer studies on HPV prevalence in men have been conducted compared to females and many studies on HPV in men have focused on high-risk populations such as men who have sex with men (MSM).[8] High vaccine coverage among girls also reduces the risk of HPV infection in boys (with the exception of MSM), so WHO considers boys and older males as secondary target populations and recommends vaccination in these groups when it is feasible and affordable.[9] There is no clinical indication for HPV testing of male genital specimens, but surveillance is important to understand the natural history of HPV and the impact of vaccination programs on males.

A variety of collection methods have been used to sample male genitals. Given that most of the male external genitalia are covered by cutaneous tissues, a method is needed to dislodge cells. Gentle abrasion of the surface can be achieved with emery paper, but a dry cotton or Dacron swab is generally less intimidating to participants. In most situations, participants are instructed on how to self-collect specimens from the glans, corona, and shaft of the penis. Instructions should be given verbally, written, and with diagrams. A private area with hand-washing facilities is required. An example protocol (Equipment and supplies, Procedure) follows:

4.7.1 Equipment and supplies

- Refrigerator (4°C, range 2°C to 8°C)
- Aptima Multitest Swab Specimen Collection Kit
- Gloves, laboratory coats, protective eyewear
- Specimen labels
- Specimen rack or device to hold tube upright after collection
- Soap, warm water, and paper towels
- Written instructions with visual aids for critical steps

4.7.2 Procedure

1. Study personnel should assemble all materials required to collect specimen. They should label collection vial with subject ID, date and other information required by study/surveillance protocol. Study personnel should verify participants identity and then explain the procedure, provide the collection kit.

2. Study participants should be given the collection kit, instructions, and directed to the private area for collection.

3. PARTICIPANT INSTRUCTIONS:

    a. You may want to partially undress before starting specimen collection.

    a. After required disrobing, wash hands with soap and water before collection.

    b. Twist open the top of the short tube with liquid, and place it on the clean surface.

c. Remove the swab from the package. Do not touch the soft tip of the swab.

d. Use the soft tip of the swab to firmly rub all surfaces of the penis (top surface, both sides and under surface) at least 3-4 times. If uncircumcised, pull the foreskin back before swabbing. This process will take about 10 seconds.

e. Carefully place the swab into a short tube with liquid, bend and break the swab shaft at the score line against the side of the tube, and close top of the collection tube tightly. Discard the shaft into designated trash area.

f. Place the collection tube in the designated area in the private room, wash hands with soap and water, and replace clothing.

g. Notify study personnel of any problems during collection, such as spilling, dropping, or touching swab. Recollection may be needed.

4. Health-care professional should make sure that the top of the collection tube is tightly closed to prevent leakage during shipment.

5. Store specimens in refrigerator (2°C to 8°C) and ship with cold packs to laboratory for HPV testing. Specimens may be stored at refrigerator for up to 60 days prior to testing or at -15°C to -82°C for long term storage. Ship under conditions maintaining storage temperature.

6. If recollection is indicated due to a problem during collection, the instructions may be reviewed with the participant and another collection kit given immediately.

4.8 ANAL SWAB SPECIMENS

Persons living with HIV, MSM and other groups such as solid-organ transplant recipients undergoing immune suppression, and women with a history of vulvar or cervical precancer or cancer, are at high risk of anal cancer. The recent publication from the Anal Cancer–HSIL Outcomes Research (ANCHOR) trial showed that treatment of high-grade anal squamous intraepithelial lesions (aHSIL) can reduce the incidence of anal cancer in persons living with HIV.[10] This has raised the question of whether HPV testing could be used as a primary or triage test in anal cancer screening.[11] While approval of clinical HPV testing in anal specimens is pending in most countries, anal specimens have been used in surveillance and research studies, generally among populations at high risk of anal cancer. Specimens collected for testing of non-HPV sexually transmitted infections are generally appropriate for HPV testing. These are often collected by health care personnel but may be self-collected. Specimens should represent the anal verge, where keratinized and glandular mucosa meet, as this is the site where most aHSIL is detected. Self-collection requires clear instructions. Instructions should be given verbally, written, and with diagrams. An example protocol (Equipment and supplies, Procedure) for self-collection follows:

4.8.1 Equipment and supplies

- Refrigerator (4°C, range 2°C to 8°C)
- Aptima Multitest Swab specimen collection kit
- Gloves, laboratory coats, protective eyewear
- Specimen labels
- Soap, warm water, and paper towels
- Written instructions with visual aids for critical steps

4.8.2 Procedure

1. Study personnel should assemble all materials required to collect the specimen. They should label collection vial with subject ID, date and other information required by study/surveillance protocol. Study personnel should verify participants identity and then explain the procedure, provide the collection kit.
2. Study participants should be given the collection kit, written instructions, and directed to the private area for collection.
3. PARTICIPANT INSTRUCTIONS:
    a. You may want to partially undress before starting specimen collection.
    b. After the required disrobing, wash hands with soap and water before collection.
    c. Unscrew the cap of the collection tube with liquid and place it on the clean surface.
    d. Remove the swab from the package. Do not touch the soft tip of the swab. Hold the swab by placing thumb and forefinger in the middle of the swab shaft covering the score line.
    e. Carefully insert the soft tip of the swab to into anus about 1-2 inches (3-5 cm) past the outside of the anus and gently rotate the swab for 5-10 seconds.
    f. Place the swab into a collection tube with liquid, bend and break the swab shaft at the score line against the side of the tube, and close top of the collection tube tightly.
    g. Place the collection tube in the designated area in the bathroom, wash hands with soap and water and replace clothing.
    h. Notify study personnel of any problems during collection, such as spilling, dropping, or touching swab. Recollection may be needed.
4. Health-care professional should make sure that the top of the collection tube is tightly closed to prevent leakage during shipment.
5. Store specimens in refrigerator (2°C to 8°C) and ship with cold packs to laboratory for HPV testing. Specimens may be stored at refrigerator for up to 60 days prior to testing or at -15°C to -82°C for long term storage. Ship under conditions maintaining storage temperature.
6. If recollection is indicated due to a problem during collection, the instructions may be reviewed with the participant and another collection kit given immediately.

4.9 ORAL RINSE SPECIMENS

Approximately two thirds of oropharyngeal cancers are HPV positive, predominately HPV16, and the increase in oropharyngeal cancers has been attributed to HPV.[2, 12] Currently there is no method to screen for oropharyngeal cancer and precancers in this anatomic region are rarely identified. HPV testing of oropharyngeal cancers is used clinically as HPV-positive tumors are associated with a better prognosis.

Oral rinse specimens are an indirect indication of oropharyngeal HPV status and are used in research and surveillance studies. A prior study found that oral rinse detects more HPV than brushes of lesions or mouth.[13] Saline or mouthwash has been used to collect specimens, generally with several 'swish and gargle' cycles. In a small study, Scope mouthwash was found to perform better than other mouthwashes and saline for DNA yield and stability.[14] Access to a sink is helpful. An example protocol (Equipment and supplies, Procedure) follows:

4.9.1 Equipment and supplies

- Refrigerator (4°C, range 2°C to 8°C)
- Sterile specimen collection cup
- Scope mouthwash
- Sterile saline solution (0.9% sodium chloride solution)
- 15-mL conical screw-on cap centrifuge tube
- Gloves, laboratory coats, protective eyewear
- Specimen labels
- Written instructions with visual aids for critical steps

4.9.2 Procedure

1. Study personnel should use new pair of gloves before working with new study participants. Assemble all materials required to collect specimen. Label collection vial with subject ID, date and other information required by study/surveillance protocol.

2. Study personnel should verify participants identity and then explain the procedure, provide the collection kit.

3. Pour 10 mL of Scope mouthwash (or saline) to the specimen collection cup without touching the rim of the cup. Saline may be used instead of mouthwash if study participants have sores in their mouth.

4. Give the cup of mouthwash to the participant and remind them not to swallow.

5. Tell participants to take the mouth wash and start swishing. Instruct participants when to swish, gargle, and spit. Swish-gargle cycle should be done every 5 seconds for three times followed by a final spit into the collection cup.

6. Take the collection cup from the participants and carefully secure the top to prevent leakage.

7. Process the specimen in the laboratory by transferring oral rinse from the collection cup to appropriately labeled 15-mL conical screw-on cap centrifuge tube. Make sure that the cap is tightly secured to prevent leakage during shipment.

8. Store specimens in refrigerator (2°C to 8°C) until they are shipped with cold packs to laboratory for HPV testing. Oral rinse in Scope may be stored at 2°C to 8°C for up to 3 weeks.

9. For long-term storage at -60°C to -80°C, specimens should be stored as cell pellets. To prepare more than one cell pellet per specimen, the 15-mL tube should be inverted 3 to 5 times to suspend cells in medium before transferring aliquots into additional tubes. Centrifuge the oral rinse specimen at 4000 x g for 10 minutes. Remove supernatant and then freeze pellets.

10. Whether shipping refrigerated oral rinse or frozen cell pellet, ship under conditions maintaining storage temperature. Scope contains 8% alcohol and require shipping methods for flammable materials.

4.10 FORMALIN-FIXED PARAFFIN EMBEDDED SPECIMENS

Residual material from archived diagnostic formalin-fixed paraffin-embedded (FFPE) blocks may be used for HPV testing as it allows histological confirmation of the specimens being tested. Routine histology processing varies in the extent of fixation and DNA/RNA preservation. Formalin fixation results in cross-links and fragmented DNA. Despite this limitation, FFPE tissues have been widely used to determine the presence and distribution of HPV types in pre-cancers and cancers[15, 16] as DNA, while fragmented, is stable for many years in the tissue block.

Pathology review is required for selection of the best block for HPV testing. Blocks should be selected based on representative histology, highest ratio of tumor/lesion to non-tumor/non-lesion tissue in block, minimal necrosis, best preservation, and sufficient residual tissue that allows collection of at least six 5-µm sections. Blocks from biopsies are preferred to those from resection specimens as they are generally better fixed.

Selected blocks are sectioned using the 'sandwich method', that is using the first and last sections for hematoxylin and eosin staining (H&E) to confirm that the intervening unstained sections (2 to 5) have the lesion of interest. This is particularly important for precancer lesions that may be quite small. The sectioning needs to be done by a histologist familiar with precautions needed to prevent polymerase chain reaction (PCR) contamination and should be prepared by trained lab personnel following standards protocol for tissue sectioning. Sections should be tested by PCR only if lesion is identified in both first and last H& E sections. If tissue is minute, Tube 1 and Tube 2 specimens collected for PCR (see below) may need to be pooled. It should be kept in mind that DNA is less stable once thin sections are cut from the block, presumably due to larger surface area available for oxidation.

In most instances, HPV testing on FFPE tissues is performed for research or surveillance. The exception is for some tumors, like oropharyngeal cancer, where the HPV status of the tumor has prognostic significance. When applied clinically, careful assay validation and calibration to expected clinical standards is required. An example protocol (Equipment and supplies, Procedure) follows:

4.10.1 Equipment and supplies

- Refrigerator (4°C, range 2°C to 8°C)
- Gloves, laboratory coats, protective eyewear
- Microtome
- Disposable low profile microtome blades
- Disposable wooden applicators
- Slide cover slip
- 3x1 inch, 1-mm thick microscope slides
- Slide box
- 2-mL conical sterile screw cap MicrewTubes with tether caps
- Specimen labels

4.10.2 Procedure

1. Lab personnel should assemble all materials required to collect specimen. They should label slides and MicrewTubes with subject ID, date and other information required by study protocol.

2. Wear gloves when cutting blocks and handling slides.

3. Clean microtome and area around cutting station with 70% alcohol before starting sectioning.

4. Use a new disposable microtome blade for each block to minimize cross-contamination of specimens.

5. Always use a new disposal wooden applicator and directly collect sections to be used for PCR from the microtome blade. Do not touch sections with gloves and the sections should not touch water bath.

6. Cut six 5-µm serial sections and distribute as follows.

   Section 1: This will be slide 1 and should be used for H&E staining for histological evaluation.

   Section 2 and 3: Place both sections in one tube (Tube 1) and the specimen should be used for DNA extraction for HPV typing.

   Section 4 and 5: Place both sections in one tube (Tube 2) and the specimen should be used for DNA extraction for HPV typing.

   Section 6: This will be slide 2 and should be used for H&E staining for histological evaluation.

7. Lab personnel should make sure that the cap of Tube 1 and Tube 2 is tightly closed, and slides are stored in slide box.

8. Ship specimens at room temperature to laboratory for histological evaluation and HPV testing. Store glass slides and specimen tubes at room temperature. FFPE specimens for HPV testing should be stored in refrigerator (2°C to 8°C) for long term storage.

4.11 SERUM

Serum (and sometime plasma) is used to document the immune response to HPV infection or HPV vaccination. The serology response does not provide information about the participants current HPV infection status at any anatomic site. Standard methods for collection of peripheral blood and serum are appropriate for HPV serology. Venipuncture should only be performed by qualified personnel trained in the technique. Whole blood should not be frozen. Blood can be stored at 2°C to 8°C for up to 24 hours before the serum is separated. If centrifuge is not available, blood should be kept refrigerated until there is complete retraction of the clot from the serum, and serum carefully removed as described below following centrifugation. An example protocol (Equipment and supplies, Procedure) follows:

4.11.1 Equipment and supplies

- Centrifuges with horizontal rotor and buckets with aerosol barriers to vacutainer tubes
- Refrigerator (4°C, range 2°C to 8°C)
- Freezer, frost free (-20°C, range -15°C -32°C)
- Tourniquet
- Sterilizing swabs/wipes
- Specimen labels
- Screw-cap cryovials for specimen storage (glass cannot be used for freezing serum)
- 5 ml vacutainer tube (no anticoagulant) with 23-gauge needle
- Sterile transfer pipettes
- Gloves, laboratory coats, protective eyewear
- Biohazard container for disposal of sharps
- Racks for vacutainer tubes and serum storage vials

4.11.2 Procedures

1. Assemble all materials required to collect blood. Label vacutainer tube with subject ID, date and other information required by study/surveillance protocol.

2. Explain procedure to subject and verify their identity. Seat subject in position that allows comfortable access to antecubital fossa.

3. Sterilize antecubital fossa with alcohol wipe. Place and tighten tourniquet. Insert needle in vein, apply vacutainer and release tourniquet as blood enters tube. When full volume is collected, remove vacutainer and needle. Apply pressure over puncture site with gauze and place band aid.

4. Discard needle into sharps biohazard bag. Discard used wipes and gauze into biohazard container.

5. Place vacutainer tube in rack and allow clot to form for 30 minutes. Centrifuge at 1000 x g for 10 minutes to separate the serum.

6. Carefully remove the serum with transfer pipette, avoiding extracting red cells, and transfer aseptically to one or more sterile labelled serum storage vials (screw capped).

7. Store serum in refrigerator (4°C, range 2°C to 8°C) for a maximum period of seven days, and ship to the laboratory on wet ice. For longer storage, serum must be frozen at -20ºC (range -15°C -32°C) and transported to the testing laboratory on frozen ice packs. Repeated freezing and thawing can have detrimental effects on the stability of antibodies, therefore at least two storage aliquots are recommended, and aliquot size should be small enough to minimize number of freeze-thaws.

4.12 SHIPPING SPECIMENS

Specimens must be shipped under conditions that protect specimen integrity for testing and, equally important, protect the safety of all who encounter the shipment. There are correct procedures to be followed depending on the material to be transported and the method of transport (air or ground). Shipping biologic specimens requires planning and coordination with the shipping company and the receiving laboratory to assure that specimens arrive and can be handled in a timely manner. Personnel involved in shipping must be trained in the appropriate methods for packing and labelling shipments, and International Aviation and Transportation Administration (IATA) certification is recommended. Unless specimens have been treated to inactivate all infectious agents, they must be handled as infectious.

Laboratories should follow the most current WHO guidance on regulations for transport of infectious substances. The shipping instructions outlined here follows the 2021–2022 WHO guidance.[17] The document includes detailed definitions and instructions for all steps required for safe transport of biologic specimens. Most of the specimens expected to be received by, or sent from, an HPV laboratory would be considered patient specimens. WHO guidance defines patient specimens as materials collected from humans or animals collected for research and/or diagnostic investigations and includes, but is not limited to, excreta, secreta, blood/blood products, tissues or body parts collected in containers, on swabs or collected in preservative medium.

HPV is a human pathogen but falls under category B infectious substance. WHO guidance classifies infectious substance as category B when it contains biological agents capable of causing infection in humans or animal but is not able to cause permanent disability or life-threating conditions or fatal disease in healthy humans or animals. According to United Nations (UN) Model regulations that outlines details packaging requirements of various class of dangerous goods, category B infectious substance such as HPV should follow packing instructions P650. Detailed

instructions for P650 can be found in WHO guidance.[17] Packaging specimens in accordance with P650 should include basic triple packaging requirements along with other stipulations outlined below. Packaging should comply with other regulations if dangerous goods such as dry ice is used in the package. If the package will cross international boarders, customs documentation should be prepared in advance of the shipment and included with the package. Regulations for shipment of infectious substance may vary depending on the origin and destination and shipper should comply with international, national, and local regulations, for example the destination country may require an import permit and declarations about the samples (e.g. what exotic infectious agents may be present). Where IATA regulations apply, those packaging the specimens must complete IATA training every 2 years.

- Triple packaging is designed to contain infectious substance during transport and should contain three layers: primary, secondary, and outer layer.

- Primary receptacle should be watertight and leakproof and must contain appropriate label describing the content. The primary receptacle must be compatible with preservation media used in specimen and should be wrapped with enough absorbent material to contain liquid in the event of breakage or leakage.

- The secondary layer should be a container that is watertight and leakproof that is used to enclose the adsorbent wrapped primary receptacle. Multiple primary receptacles can be placed in the same secondary container if the substances belong to the same dangerous goods class. If the primary receptacles are fragile, each one must be individually wrapped or cushioned to prevent them from contacting each other.

- Outer layer should be a container of appropriate strength and dimension so that secondary container can be protected from physical damage during shipment. Outer container must have at least one surface with minimum dimension of 100 mm x 100 mm. Place documentation between the secondary container and outer layer.

- For surface transport via road, rail or maritime, either secondary or outer layer must be rigid. The air transport requires rigid outer layer. There are no limits on quantity of material being shipped via surface transport. For air transport, primary receptacle must not contain more than 1 liter and outer layer container must not contain more than 4 liters of material (excludes any quantity of dry ice or liquid nitrogen).

- The primary receptacle or secondary container must withstand internal pressure of 95kPa. The complete triple package should be appropriate strength and quality and must pass a 1.2-meter drop test.

- The complete triple package must be labeled **UN3373 Infectious substance Category B** on the outer layer.

- In addition to the triple packaging, infectious substances may need preservatives like coolants or stabilizers to maintain integrity during transport. Coolants, such as dry ice and liquid nitrogen, keep the temperature low but are considered dangerous goods and have specific packaging requirements. Stabilizers, like sorbitol and formaldehyde, prevent degradation and must be added to the primary receptacle. Both coolants and stabilizers require proper handling, marking, and documentation.

A shipping log detailing the specimen IDs included in the shipment should be included in the package. In some instances, a temperature logger may be included to monitor temperature

fluctuations during transit. In addition, a copy of the shipping log should be retained by the laboratory or group originating the shipment. If the internal review board (IRB) protocol or regulations specify participant privacy, the staff preparing the shipment should confirm no personal identifiable information is listed on the specimens or documentation. The receiving laboratory should be notified by the time the shipment is made. This notification should include the name and contact information for one or more personnel responsible for initiating the shipment, the transport company and contact information, the shipment tracking number, the expected date of arrival, an electronic copy of the shipping log, and if applicable a copy of the customs documents.

4.13 RECEIVING SPECIMENS AND RECORDING

Specimen shipments must be unpacked as soon as possible after they are received by the laboratory. If unpacking must be delayed for some reason, shipments should be stored in a refrigerated area (2-8°C) until they can be handled. Shipments should be unpacked in a designated area located away from other laboratory activities. As there is always the possibility of breakage and leakage of specimens, the workbench in the unpacking area must be clean and have a surface covering that is easily disinfected using common laboratory disinfectants (70% ethanol, sodium hypochlorite solution, etc.). The workbench should be equipped with a discard container, alcohol swabs and paper towels. The external surface of the package should be inspected for any physical damage due to water or crushing. As the package is opened, the condition of any packing coolants (cold packs, wet or dry ice) should be noted. Unpacking is ideally handled by two people: one gloved to open the package; check for leakage, breakage, or contamination of documents; and to handle the specimens; and the other to record the specimens and note those that were damaged. Serum, specimens for DNA extraction, or DNA extracts should be frozen -20°C (range -15°C -32°C) unless testing is to be performed within a day or two, in which case specimens may be refrigerated at 4°C (range 2°C to 8°C).

Each specimen must be matched against the shipping log to be sure that all specimens were received and that no additional specimens were included. When possible, barcodes on specimen labels should be scanned into an electronic copy of the shipping log to cross-check the labels against the log. As soon as the shipment inventory is completed, personnel responsible for initiating the shipment should be notified that it has been received and if there were any problems noted with it (insufficient coolant, discrepancies between the specimens in the shipment and the shipping log, damaged specimens).

The receiving laboratory should create a database for received specimens. This should include:

- Study or reason for submission of specimen (project or study identification)
- Subject identification (in a standard format, usually alphanumeric, often including age and sex)
- Specimen identification number (used for all aliquots and recording results)
- Date of receipt of specimen
- Date of specimen collection
- Specimen type (swab, biopsy, serum, etc.)
- Volume of specimen
- Diagnosis, with date of diagnosis (if available and appropriate)
- Residence of subject (as appropriate)
- Storage location (unless testing is performed immediately)
- Comment (note any problems with specimen)

4.14 REFERENCES

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