


<b>Title:</b>	Collection, Processing and Storage of Female Genital Secretions		
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### 1 Purpose

This Standard Operating Procedure (SOP) describes procedures for the collection, processing and storage of samples obtained from the female genital tract for ACTG and IMPAACT studies.

### 2 Scope

Users of the ACTG/IMPAACT Lab Manual

### 3 Background

The first studies of genital HIV-1 infection used viral cultures to provide quantitative evidence for the infectiousness of female genital secretions as a mechanism for the sexual transmission of HIV-1<sup>13.1</sup>. Subsequent studies have used polymerase chain reaction amplification of HIV-1 nucleic acids to quantify HIV-1 and suggested that higher genital tract HIV-1 levels were likely a measure of increased infectiousness<sup>13.2</sup>.

Many studies have measured HIV-1 concentrations in cervicovaginal fluid to assess the infectiousness of HIV-1 transmitted sexually<sup>13.3</sup> and perinatally<sup>13.4</sup>. Moreover, sexual transmission is likely enhanced by factors that increase inflammation of genital mucosa and thus genital HIV-1 levels<sup>13.5-13.7</sup> and decreased by ART, which decreased genital HIV-1<sup>13.8-13.9</sup>. A recent prospective study showed that higher female genital HIV-1 RNA level independently predicted HIV-1 transmission risk after adjusting for plasma HIV-1 levels<sup>13.10</sup>.

Compartmentalization of HIV-1 between the female genital tract and blood has been observed by statistical methods and phylogenetic tree topologies; however, the detection of monotypic sequences across the cervix and blood suggests that cells with provirus home to the cervix and undergo clonal expansion within the cervix; as such, the appearance of viral compartmentalization in the female genital tract results from burst of viral replication and is not due to unique viruses that evolve within the genital tract<sup>13.11-13.12</sup>. Clearly, the sampling of the female genital tract for HIV-1 is complicated and requires standardized methods for sample collection, including fluid and potentially tissue biopsy.

In addition, interest in penetration of antiretrovirals [and other medications taken by HIV+ participants] has expanded, especially in the context of new strategies for pre-exposure prophylaxis<sup>13.13-13.14</sup>. To assure accurate and optimized results, standardized methods are warranted but in many cases have not been established. Therefore, the collection, processing and storage of female genital tract samples for pharmacology must be provided by the laboratory assigned to perform the test or the protocol pharmacologist if the assignment has not been determined.

The ACTG and IMPAACT networks have established standard operating procedures (SOPs) for the collection of female genital tract fluid for HIV-1 RNA quantification. This document includes the SOPs for the collection of endocervical canal fluid (wicking fluid with Tear-Flow™ strips), endocervical cells (Cytobrush), direct aspiration of cervicovaginal fluid and collection of ectocervicovaginal lavage fluid (CVL). These procedures have been in wide use over the past several years and have been used to support the study of female genital tract evaluations for HIV-1 RNA and cytokines for several ACTG protocols. A cervical punch biopsy method has also been developed but not validated by the ACTG at the time of this writing.

## 4 Budgetary Considerations

- 4.1 Supplies
- 4.2 Facilities
- 4.3 Approximate participant reimbursement (range provided in protocol budget)
- 4.4 Sample analyses (will vary with the protocol)

## 5 Authority and Responsibility

- 5.1 The Network Laboratory Directors (or his/her designee), in conjunction with the CURE Transformative Science Group, have the authority to establish, review and update this procedure.
- 5.2 The ACTG/IMPAACT Laboratory Technologist Committee (LTC) is responsible for the maintenance and control of SOP documentation.
- 5.3 The Laboratory Director is responsible for the implementation of this LTC SOP or laboratory-specific SOP and for ensuring that all appropriate personnel are trained. A laboratory SOP must:
  - 5.3.1 Include, without procedural modification, the portions of the current version of the LTC SOP that are used within the network site-affiliated laboratory.
  - 5.3.2 Reference the current version of the LTC SOP.
- 5.4 All laboratory technicians are responsible for reading and understanding this SOP prior to performing the specimen processing procedures described herein.
- 5.5 The site PI and designees are responsible for understanding and adhering to the participant preparation and specimen collection components.

## 6 Eligibility Requirements

- 6.1 Inclusion criteria
  - 6.1.1 Able to provide informed consent.
  - 6.1.2 For females of reproductive potential (defined as girls who have reached menarche and women who have not been post-menopausal for at least 24 consecutive months, i.e., who have had menses within the preceding 24 months).
  - 6.1.3 A negative pregnancy test within 3 days prior to sampling.
- 6.2 Exclusion criteria
  - 6.2.1 Having undergone surgical sterilization [e.g., hysterectomy, bilateral oophorectomy tubal ligation or salpingectomy]
  - 6.2.1 Known abnormalities of the vaginal mucosa or significant vaginal symptoms, which, in the opinion of the study investigator, represent a contraindication to genital sampling. This includes but is not limited to pelvic surgery within the 3 months prior to entry.
  - 6.2.2 Inability or unwillingness for candidates to avoid intravaginal products or other insertions for 48 hours before genital secretion sampling.

- 6.2.3 Menses may be inhibitory to molecular assays and should be addressed in the individual protocol.

## 7 Equipment, Consumables and PPE

Some current pharmacology specimen collection and processing options: analytical balance (to pre-weigh and post-weigh cryogenic vials with, and without specimen, respectively), and collection device with container (soft-cup, wicks, Dacron polyester swabs) and rovimeters (for aspirated collections of cervicovaginal fluid). The protocol pharmacist and/or laboratory performing the test should be consulted for the protocol-specific pharmacology specimen information.

- 7.1 Speculum
- 7.2 Forceps: Tenaculum, Baby Tischler
- 7.3 Wicks: Tear-Flo™ Strips: Wilson Ophthalmic at (800) 222-2020, product # 060-000002-00, 100 strips/box
- 7.4 Swabs, Dacron polyester, sterile, individually wrapped. Recommend flocked. Must use consistent swabs for entire study.
- 7.5 Fluid aspirator (for cervicovaginal fluid): e.g. CarTika Medical, Inc. P/N 10023
- 7.6 Polypropylene cryovials, e.g. SARSTEDT Screw cap micro tube, external thread #72.694.006 or Screw cap micro tube, conical, #72.692.005
- 7.7 Sterile 10mL syringe
- 7.8 Sterile plastic transfer pipette or 14-gauge angiocath
- 7.9 15mL disposable centrifuge tube, sterile, conical bottom, graduated polypropylene
- 7.10 Cytobrush collection kits for protocols which require and specify Digene collection kits (Note: Digene collection kits are typically used for HPV measurements; cytobrushes for collecting samples for pathogens other than HPV, including HIV, can generally be found in the local clinic):  
  
Digene collection kits for cytobrush specimens for some protocols may be provided centrally. If the kits are not provided by the protocol, they may be purchased directly from Digene, product number 5126-1220
- 7.11 Appropriate PPE for participant specimen collection and for specimen processing.

## 8 Reagents and Reagent Preparation

- 8.1 Normal saline, sterile, for CVL collection
- 8.2 Sterile Phosphate Buffered Saline
- 8.3 Cryopreservation media (90% Fetal bovine serum, 10% DMSO)

## 9 Procedure: Participant Preparation and Specimen Collection

The subject must refrain from any kind of sexual activity, douching, and inserting any intravaginal products for at least 48 hours prior to the collection of vaginal/cervical specimens. The participant should undress from the waist down and lie on her back on the exam table for collection of these

samples in the clinic. For some protocols, vaginal swabs may be collected at home; specific instructions will be provided in the protocol Manual of Operations (MOP).

Samples will be collected by the following methods in the following order: 1) endocervical wicks (Tear-Flo™), 2) cervicovaginal fluid aspiration, 3) vaginal swab, 4) cervicovaginal lavage (CVL), 5) cervical swab, 6) endocervical cytobrush, 7) Pap test, and 8) cervical or vaginal biopsy (or both), if being collected.

### 9.1 Endocervical canal fluid collected by Tear-Flo™ strip wicking.

The purpose of this collection procedure is to obtain endocervical canal fluid for viral RNA quantification.

9.1.1 Gently insert an unlubricated speculum into the vagina.

9.1.2 Two Tear-Flo™ strips will be used as wicks to collect primarily cell-free virions from the endocervical fluid. If excess mucus or menses clot has accumulated near the cervical os, a large cotton-tipped swab may be used to gently remove this material before inserting the strips.

9.1.3 Sample collection using Tear-Flo™ strips (see Fig 1 below):

9.1.3.1 Please take careful note that, TWO (2) Tear-Flo™ strips should be used simultaneously.

9.1.3.2 Use forceps (ring or sponge forceps work well) to hold two Tear-Flo™ strips on the squared end and gently insert the two strips simultaneously into the vagina, placing through the cervical os into the distal endocervical canal and hold in place to adsorb sample.

9.1.3.3 Each Tear-Flo™ strip adsorbs approximately 12µl of specimen. Adsorption usually takes approximately one minute, but may require a little more time.

9.1.3.4 Place the round end of the two strips over and slightly inside one labeled 2mL cryovial. Cut the strips at the “15” mark with clean scissors, allowing the round end to fall into the cryovial. Securely cap the cryovial. , Discard the square end after cutting.

9.1.3.5 Send the sealed vial to the local laboratory for processing.

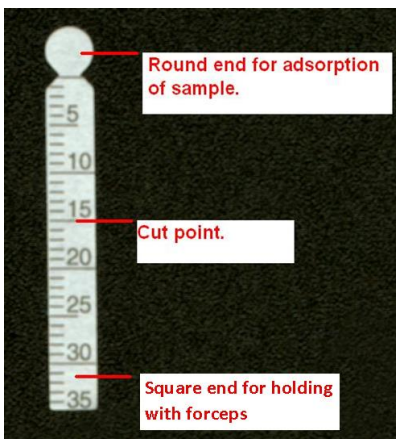
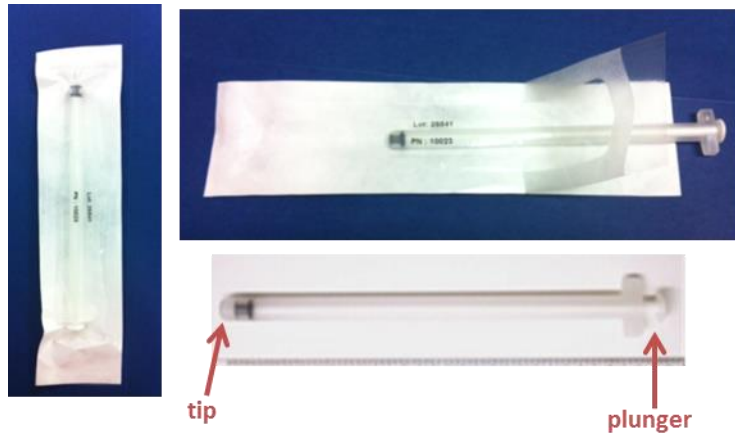


Figure 1. Tear-Flo Strip

### 9.2 Vaginal aspirate of ectocervicovaginal fluid

The participant should undress from the waist down and lie on her back on the exam table for approximately 15 minutes prior to beginning collection of this sample.

- 9.2.1 Partially peel the aspirator envelope open, exposing the plunger.



- 9.2.2 Put on clean gloves.
- 9.2.3 Remove the aspirator from the package. Do not discard the package (the aspirator may be returned to the package after sample collection). Pull the plunger away from the tip of the aspirator and then push it back towards the tip. Do this a few times to loosen the plunger and prevent it from sticking. When done, push the plunger all the way down, towards the tip.
- 9.2.4 While separating the labia with one hand, use the other hand to hold the plunger-end of the aspirator between the thumb and forefinger. Insert the rounded end (tip) of the aspirator into the participant's vagina until it touches the back of the posterior fornix (imagine trying to reach the area below the cervix). Pull the entire aspirator out ever so slightly to avoid suction of tissue.



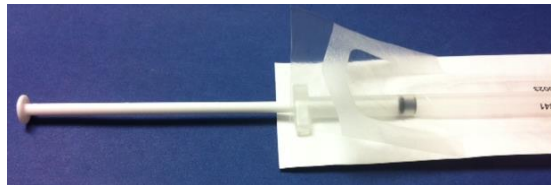
Note: Once the aspirator is inserted completely into the vagina, the labia do not need to be separated.

- 9.2.5 Hold the aspirator in place with one hand, and using your other hand, very slowly pull the plunger out of the aspirator (away from the tip). This will cause vaginal fluid to be drawn into the aspirator. Continue to pull the plunger out with one hand while removing the aspirator from the vagina with the other hand.



Slowly pull plunger  
away from tip

- 9.2.6 Using the graduation marks on the barrel of the aspirator, determine the volume of fluid that was collected. Be sure that air bubbles have been eliminated before reading the volume. A minimum of 200  $\mu$ L of fluid should be collected. If the aspirator did not collect enough vaginal fluid with the first collection attempt, the aspiration may be repeated.
- 9.2.7 If the sample will be transported to the laboratory to be dispensed into a cryovial, carefully slide the aspirator back into the envelope, tip first, without touching the outside of the envelope. Be careful that the tip of the aspirator does not touch anything as it is put back into the envelope. The plunger should remain extended.



- 9.2.8 The sample may also be dispensed into the cryovial immediately after collection. To do so, dispense the fluid into a sterile, screw top 1.8-2.0mL cryovial by slowly pushing the plunger all the way down toward the tip. Take care that the tip of the aspirator does not touch anything other than the inside of the sterile tube before the sample is dispensed.
- 9.2.9 Deliver the sample to the laboratory for storage at  $-70^{\circ}\text{C}$  until shipment (refer to the Laboratory Processing Chart [LPC]).
- 9.3 Vaginal Swab
- 9.3.1 Insert a Dacron swab gently and rotate 360 degrees in all four quadrants of the vaginal vault.
- 9.3.2 Place the swab into a sterile 2mL cryovial. Break or cut the shaft short enough to fit in the cryovial and allow the cap to be tightly sealed.
- 9.3.3 Specimens should be transported to the laboratory within one hour. If this is not possible, place the specimen on wet ice or refrigerate at  $4^{\circ}\text{C}$  until transport, up to 4 hours. If the vial is to be placed on wet ice, seal the cryovial in a plastic baggie or equivalent to keep exterior of vial dry.



- 9.4 Ectocervicovaginal lavage (CVL). The purpose of this collection procedure is to obtain a washing of virus and cells from the ectocervix and fluid from the posterior vaginal fornix for viral and immunologic studies.
- 9.4.1 Draw up 10mL of either nonbacteriostatic normal saline (saline for irrigation) or 1X phosphate buffered saline in a 10mL syringe.
  - 9.4.2 Use clean scissors to cut a sterile plastic transfer pipette just below the bulb. Discard the bulb and place the pipette tip on the syringe. Alternatively, a 14-gauge angiocath can be inserted over the tip of a 10mL syringe. It may be helpful to seal the junction with parafilm.
  - 9.4.3 Introduce the syringe through the speculum to the opening of the cervical os, but do not insert into the os.
  - 9.4.4 Aim a continuous stream of saline directly at and into the os to bathe the cervix and the ectocervix.
  - 9.4.5 Allow the fluid to pool into the posterior fornix and aspirate into the same syringe.
  - 9.4.6 Repeat this procedure exactly 5 times with the same fluid; do not add any additional saline or PBS to the wash.
  - 9.4.7 Aspirate and transfer the fluid to a sterile 15mL conical polypropylene tube.
  - 9.4.8 Transport to the laboratory within 1 hour of collection. If this is not possible, place specimens on wet ice or refrigerate at 4°C until transport, up to 4 hours. If the tube is to be placed on wet ice, seal the tube in a plastic baggie, or equivalent, to keep exterior dry.
- 9.5 Endocervical Swab
- 9.5.1 Gently insert a Dacron swab 1cm into the cervical os and rotate 360 degrees.
  - 9.5.2 Place the swab into a sterile cryovial. Break or cut the shaft short enough to fit inside the cryovial and allow the cap to be tightly sealed.
  - 9.5.3 Specimens should be transported to the laboratory with one hour. If this is not possible, place the specimen on wet ice or refrigerate at 4°C until transport, up to 4 hours. If the vial is to be placed on wet ice, seal the cryovial in a plastic baggie, or equivalent, to keep exterior of vial dry.
- 9.6 Endocervical canal cytobrush.
- The purpose of this collection procedure is to obtain primarily cells for viral DNA quantification.
- Please note that the collection media listed below are protocol specific and are NOT interchangeable.
- 9.6.1 Gently insert a cytobrush with a plastic shaft 1cm into the cervical os and rotate exactly 360 degrees. Note: bleeding usually occurs with the cytobrush.
  - 9.6.2 The cytobrush must be placed in the appropriate protocol- specified collection and transport system which could be an empty sterile cryovial or a Digene collection vial.
  - 9.6.3 Snap off the end of the cytobrush so that the brush portion can fit within the transport tube/cryovial. The cytobrush shaft should snap easily, particularly if it is scored with a pair of clean scissors approximately 2cm from the brush end of the handle.

- 9.6.4 Place the brush in the vial, being certain that the brush is immersed in the Digene transport medium (if applicable), and so that the scored area is approximately even with the lip of the vial. Hold the vial containing the swab upright with one hand and bend the shaft with the other hand, snapping off the top of the swab handle. Firmly tighten the lid of the cryovial.
- 9.6.5 Cytobrushes in the Digene fluid can remain at 4°C for up to 72 hours; transport to processing lab. Cytobrushes in dry cryovials should be transported to the processing lab within 4 hours and frozen within 6 hours. Clinicians should send the sealed vial to the local laboratory for processing.
- 9.7 Papanicolaou test (Pap test)
  - 9.7.1 Follow local instructions or protocol-specific instructions (if applicable) for collecting and processing Pap test specimens as part of an ACTG protocol.
- 9.8 Cervical or Vaginal Biopsy (or both)
  - 9.8.1 A cervical and/or vaginal biopsy will provide tissue for immunologic, pharmacologic, or virologic assessment. Generally, no prior preparation, such as fasting or sedation, is required for a simple cervical or vaginal biopsy. The participant can take a pain reliever 30 minutes before the procedure.
  - 9.8.2 Put participant in lithotomy position and insert a speculum to visualize the vagina and cervix.
  - 9.8.3 Spray the cervix and/or vaginal fornices with topical 20% benzocaine spray.
  - 9.8.4 Anesthetize the area of biopsy using a small needle to inject 2% lidocaine solution.
  - 9.8.5 Tenaculum forceps may be used to hold the cervix steady for the biopsy. The participant may feel some cramping when the tenaculum forceps is applied.
  - 9.8.6 The amount and location of tissue removed depends on the type of biopsy. For a simple cervical biopsy, use a Baby Tischler biopsy forceps to obtain a 3mm x 3mm x 1mm cervical tissue at the 3 or 9 o'clock position. For a vaginal biopsy, use a Baby Tischler biopsy forceps to obtain vaginal tissue from either the left or right vaginal fornix.
  - 9.8.7 When a biopsy is performed, the participant may feel a slight pinch or cramp. Bleeding from the biopsy site may be treated with a topical medication (e.g. Monsel's solution).
  - 9.8.8 Biopsy will be immediately placed in transport media as required for the intended test. Consult the protocol "ologist" and the testing laboratory.

## 10 Procedure: Laboratory Specimen processing

Note: For pharmacology specimens, contact the laboratory assigned to perform the test and/or the protocol pharmacologist for specifications. For example, centrifugation to fractionate CVL may be discouraged for PK since some drugs may bind to the mucous and proteins that would pellet

- 10.1 Endocervical canal fluid specimens collected by Tear-Flo™ strip wicking
  - 10.1.1 Log samples into LDMS (LDMS specimen code: CER/NON/TFS).
  - 10.1.2 Label and freeze upright at -70°C or colder.
  - 10.1.3 Do not open the vials; do not remove Tear-Flo™ strips from the vial.

- 10.1.4 Document in LDMS if the sample appears to contain blood.
- 10.2 Vaginal Swab
  - 10.2.1 Please note that this procedure may vary if swab is being collected for pathogens other than HIV such as Candida, BV, HPV, and other STDs.
  - 10.2.2 Log samples into LDMS (LDMS specimen code: VAG/NON/SWB)
  - 10.2.3 Do not open cryovial; do not discard swab.
  - 10.2.4 Label and freeze upright at  $-70^{\circ}\text{C}$  or colder as soon as possible.
  - 10.2.5 Document in LDMS if the sample appears to contain blood.
- 10.3 Ectocervicovaginal lavage (CVL)/Cervicovaginal lavage
  - 10.3.1 Log samples into LDMS. (LDMS specimen code will vary with processing.)
  - 10.3.2 Document the sample volume and obvious presence of gross blood or mucous in the LDMS.
  - 10.3.3 Whole CVL
    - 10.3.3.1 Vortex briefly to ensure equal concentrations in each aliquot.
    - 10.3.3.2 Prepare desired number of 1.0mL aliquots, per the LPC.
    - 10.3.3.3 Label and freeze as soon as possible at  $-70^{\circ}\text{C}$  or colder.
    - 10.3.3.4 LDMS spec code: LDMS spec code: CVL/NON/CVL
  - 10.3.4 Fractionated CVL
    - 10.3.4.1 Centrifuge CVL at 400-800 x g for 10 minutes.
    - 10.3.4.2 Aspirate the supernatant (including any mucous) and store as 1.0mL aliquots.
    - 10.3.4.3 Label and store CVL supernatant aliquots at  $-70^{\circ}\text{C}$  or colder. LDMS spec code: CVL/NON/FLD
    - 10.3.4.4 Resuspend the cell pellet in 10mL PBS.
    - 10.3.4.5 Centrifuge at 600 x g for 10 minutes.
    - 10.3.4.6 Repeat steps 10.3.3.4 and 10.3.3.5 once and resuspend cells in 1mL of PBS.
    - 10.3.4.7 Divide one-half of the cell suspension (0.5mL) between two labeled conical cryovials; i.e., 0.25mL/conical cryovial.
    - 10.3.4.8 Centrifuge these two 0.25mL cell suspensions at the highest speed in a microfuge for 3 minutes.
    - 10.3.4.9 Aspirate the supernatants and store at  $-70^{\circ}\text{C}$  or colder as dry cell pellets. LDMS spec code: CVL/NON/PEN
    - 10.3.4.10 Cryopreserve the remaining 0.5mL cell suspension. Gently centrifuge the cells at 400xg for 5 minutes. Resuspend in 1mL cryopreservation media. Divide suspension into 2 aliquots. Freeze in step-down fashion, as per PBMC cryopreservation. Recommend shipment on dry ice to testing lab or BRI within 1 month of collection for long term storage. Details should be

specified in LPC. Long term storage should be in vapor phase liquid nitrogen. LDMS spec code: CVL/NON/CLN/DMS

- 10.4 Endocervical Swab:
  - 10.4.1 Please note that this procedure may vary if swab is being collected for pathogens other than HIV such as Candida, BV, HPV, and other STDs.
  - 10.4.2 Log samples into LDMS (LDMS specimen code CER/NON/SWB)
  - 10.4.3 Do not open cryovial; do not discard swab.
  - 10.4.4 Label and store at  $-70^{\circ}\text{C}$  or colder as soon as possible.
  - 10.4.5 Document in LDMS if the sample appears to contain blood.
- 10.5 Cytobrush:
  - 10.5.1 Cytobrush in Digene transport media
    - 10.5.1.1 Log samples into LDMS (LDMS specimen code CER/NON/CTB/DTM)
    - 10.5.1.2 Do not open cryovial; do not discard cytobrush.
    - 10.5.1.3 Vortex vial for 5 seconds.
    - 10.5.1.4 Label and freeze upright at  $-70^{\circ}\text{C}$  or colder.
    - 10.5.1.5 Document in LDMS if the sample appears to contain blood.
  - 10.5.2 Cytobrush specimens in dry cryovials:
    - 10.5.2.1 Log samples into LDMS (LDMS specimen code CER/NON/CTB)
    - 10.5.2.2 Do not open cryovial; do not discard cytobrush.
    - 10.5.2.3 Label and freeze upright at  $-70^{\circ}\text{C}$  or colder.
    - 10.5.2.4 Document in LDMS if the sample appears to contain blood.

## 11 Forms

- 11.1 Informed consent example. See [Appendix A](#)

## 12 Limitations

For pharmacology specimens, the stability of each drug (or its metabolites) in each sample type (matrix) must be proven under specific parameters of collection and storage vessel, time, temperature for collection, processing and storage steps. In addition, the specificity of the method for each drug in each matrix must be proven prior employing a method in a PK laboratory. These PK assay limitations are required to be entered to the CPQA PK AVR/SOP submission utility prior to method review and approval. Once the method is approved, the method details, including specimen collection, additives, processing, storage, and sample stability are added to the DAIDS network approved PK Assay Directory. This directory is updated and posted on a monthly basis. Generally speaking, when parameters are not known, PK specimens are processed as quickly as possible and stored frozen at  $-80^{\circ}\text{C}$ . However, if a method for the drug-matrix pair for the specific analysis laboratory is NOT available, it is best to contact the CPQA at [cpqasupport@fstrf.com](mailto:cpqasupport@fstrf.com) for assistance.

## 13 Literature References

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- 13.2 Baeten et al., Curr HIV Res 2003; 1:69-86; Coombs et al., AIDS 2003; 17:455-80
- 13.3 Anderson, Cu-Uvin. Curr infect Dis Rep 2008; 10:505-11
- 13.4 Gaillard et al., AIDS 2000; 14:2341-48
- 13.5 McClelland et al., AIDS 2001; 15:105-10
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- 13.7 Baeten et al., JID 2008; 198:1804-08
- 13.8 Attia et al., AIDS 2009; 23:1397-1404
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- 13.13 Dumond et al AIDS 2007; 21(4); 1899-1907
- 13.14 Keller et al, PLoS ONE. 2011 Jan 25;6(1):e16475.

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- 14.4 ACTG/IMPAACT Laboratory Technologists Committee:
  - 14.4.1 Joan Dragavon, Research Scientist, University of Washington
  - 14.4.2 Carmen Irizarry, Laboratory Supervisor, University of Puerto Rico
  - 14.4.3 Robin DiFrancesco, CPQA Program Manager, State University of New York at Buffalo
  - 14.4.4 Melissa B. Austin, Laboratory Project Manager, HIV/AIDS Network Coordination

## Appendix A: Informed Consent Example

### SAMPLE CONSENT FORM

**This example is derived from Protocol A5240**

List study name: \_\_\_\_\_

List Site personnel Contact information: \_\_\_\_\_

#### **Researchers' statement**

We are asking you to volunteer for a research study conducted by the AIDS Clinical Trials Group (ACTG) and sponsored by the National Institutes of Health (NIH). The study will be done at \_\_\_\_\_. The purpose of this consent form is to give you the information you will need to help you decide whether or not to be in the study. Please read the form carefully. You may ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. When all of your questions have been answered, you can decide if you want to be in the study or not. This process is called "informed consent".

#### **PURPOSE**

We are asking you to join this study because you have HIV, are female, and have not had any major health problems.

This study will test \_\_\_\_\_.

Enter protocol-specific background information to explain the purpose of the study:

About \_\_\_\_\_ women will join the study.

*For A5240 example: The HPV family are common viruses that cause warts, and can also cause cervical, vulvar and anal cell growth problems (dysplasia) that can progress to cancer. There are over 90 HPV types, and of these, approximately 30 types are sexually transmitted and infect the genital and anal areas of both women and men. HPV types 16 and 18 cause most cervical dysplasia and cancer. HPV types 6 and 11 cause most genital warts, but do not generally cause cancer.*

*A new, approved HPV vaccine (GARDASIL) against HPV types 6, 11, 16 and 18 prevents HPV infection and cervical dysplasia among HIV-negative women. This vaccine has not been studied yet in HIV-positive women. HPV infection can be more severe and harder to treat in HIV-positive people. Also, vaccines may not work as well to protect HIV-positive people. For these reasons, it is important to find out how well the HPV vaccine will work to protect HIV-positive women. This study will see if the HPV vaccine can help your body make antibodies to fight off HPV, and will also see whether the vaccine is safe for HIV-positive women.*

*The vaccine is given as a 3-dose series completed over 6 months. The vaccine does not contain HPV, and you cannot get HPV by taking the vaccine. The study will last for about one and a half years.*

## STUDY PROCEDURES

### Before the Study Starts

After you have read and signed this informed consent form, you will come to the clinic for a screening visit to see if you can join the study. The screening visit should last about 1 hour. This visit will include:

- Confirmation of your HIV infection. If there is no record available, we will do another HIV test. You may have to sign a separate consent form for the HIV test.
- Complete physical exam. We will ask you questions about your medical history and medications you are taking now and have taken in the past.
- Blood draw (about \_\_\_\_teaspoons) for routine safety tests, HIV viral load (a measure of how much HIV is in your blood) and CD4+ cell counts (the number of white blood cells that fight infection).
- Pregnancy test from a urine or blood sample, if you are able to become pregnant. You will not be able to enroll in this study if you are pregnant or breast-feeding.
- Pap smear will be done if you have not had one in the past six months. A Pap test is the collection of a very small amount of cells obtained by a gentle scraping of the cervix (the opening to the womb or uterus). This is done by inserting a speculum (an instrument that opens the vagina) to see the cervix.
- Anal examination will be performed to check for anal cancers.

### Study Entry and First Vaccine

If you qualify for the study, you will return to the clinic within the next 45 days after the screening visit. Please do not have any sexual activity, and do not douche or use any vaginal or anal products for 2 days before this visit.

The study entry visit should last about 1 hour and will include:

- A brief physical exam. We will ask you questions about medications you are taking now and have taken in the past. We will also ask about your lowest ever CD4+ cell count.
- A brief mouth exam to check if you have HPV in your mouth. This involves looking at your tongue, cheeks, roof of mouth, floor of mouth, lips and gums.
- We will ask you about any health problems you have had since the screening visit.
- Blood draw (about 6 teaspoons) for routine safety tests and to determine your HIV viral load and CD4+ cell count. We will also check an HPV antibody test (substances that fight the HPV virus).
- Urine pregnancy test, if you are able to become pregnant. This test may not be necessary if you had a negative pregnancy test within 2 days of the study entry visit.
- The level of HIV viral load in the cervix will be measured by inserting a speculum into the vagina and then placing 2 filter papers inside the opening of the cervix for about one minute. Then, a cervical brush will be put in the cervix for a few seconds, to test for HPV.
- An anal swab to check for HPV DNA. This takes a few seconds.
- We will give you the first HPV vaccine by injection into your upper arm or thigh muscle
- You will stay in the clinic for at least 30 minutes after the vaccine injection, to watch for any bad reaction to the vaccine.

### Vaccine follow-up

We will call you 1-2 days after the vaccine, to see how you feel and whether you had any problems. We will ask you to come to the clinic if you have any serious reactions to the vaccine.

### During the Study

You will return for study visits at weeks 4, 8, 12, 24, 28, 52, and 72. Each visit should take about one hour. Each of these visits will include:

- Brief physical exam, including your mouth. We will ask you questions about medications you are taking now and have taken in the past.
- Blood draw (up to 6 teaspoons) for routine safety tests, CD4+ cell count, HIV viral load, and HPV antibody tests. Not all of these tests are done at every visit.
- We will ask you about any health problems you have had since your last visit.
- Urine pregnancy test, before each vaccine and at any time you think you may be pregnant.

### Vaccines 2 and 3

You will receive the HPV vaccine again at weeks 8 and 24. This will be just like the first vaccine, in the muscle of your upper arm or thigh. You will stay in the clinic for at least 30 minutes after the vaccine injection, to watch for any bad reaction to the vaccine.

We will call you 1-2 days after the vaccine, to see how you feel and whether you had any problems. We will ask you to come to the clinic if you have any serious reactions to the vaccine.

### Pelvic and anal specimen collection

Study visits at weeks 28 and 52 will also include a pelvic examination and anal swab. Please do not have any sexual activity, and do not douche or use any vaginal or anal products for 2 days before this visit.

These visits will include:

- Measurement of the HIV viral load in the cervix, by inserting a speculum into the vagina and then placing 2 filter papers inside the opening of the cervix for about one minute. Then, a cervical brush will be put in the cervix for a few seconds, to test for HPV.
- At week 52, we will also do a Pap smear and an anal exam.
- An anal swab will be collected to check for HPV DNA. This takes a few seconds.

### Additional tests

You may be one of 75 study participants who will be asked to have extra tests. If you do not want to have these extra tests done, you can still be in the study. These extra tests will take an additional 30 minutes or less at some of the study visits.

Extra tests at most study visits will include:

- About 3 tablespoons of blood for immune response (cells in the blood that fight infections).

Extra tests at the study entry visit and weeks 28 and 52 will also include the following tests for HPV:

- Two small smears collected by brushing the back of your tongue and cheek.
- You will gargle saltwater and then spit in a tube.
- You will sit for a minute without swallowing and then spit into a tube.

Extra tests at the study entry visit and at weeks 28, 52, and 72:



- If a wart is seen in your mouth during the brief oral exam, you will have a small smear collected by brushing the wart for HPV testing.

These extra blood and mouth samples will be stored with a study code, not your name, and used for future testing for this study.

#### Stopping the study early

If you wish to stop the study before you complete all visits, we will ask you to come to the clinic for a final study visit. This visit will include most of the tests listed under “Study Entry,” plus a Pap test.

#### Stopping the vaccine early

If you stop the vaccine series before the last dose at week 24, we will still ask you to come to the clinic for all the remaining study visits.

#### If you do not enroll into the study

After the screening visit, if you decide not to take part or are not able to join the study, we will still keep some of your information. Specifically, we will keep a record of your age, gender, race and some clinical and laboratory information. This will help us learn whether there are patterns or common reasons why people do not join a study. We will protect your privacy and confidentiality.

### **RISKS, STRESS, OR DISCOMFORT**

The vaccine used in this study may have side effects, some of which are listed below. These lists do not include all the side effects seen with this vaccine. These lists include the more serious or common side effects with a known or possible relationship. If you have questions concerning the additional vaccine side effects, please ask the medical staff at your site.

For your safety, you must tell the study doctor or nurse about all medications you are taking before you start the study and also before starting any new medications while on the study. Also, you must tell the study doctor or nurse before enrolling in any other clinical trials while on this study.

The risks of the study vaccine are listed below.

#### The following serious side effects have been associated with Gardasil

- Hypersensitivity (allergic) reactions, such as rash fever, flu-like feeling, blisters, facial swelling, or even problem breathing. These reactions, in severe form, may be life threatening
- Guillain-Barré Syndrome (a form of paralysis)

#### In addition to the serious side effects listed above, additional side effects include:

- Soreness, tenderness, itching, redness, bruising, or swelling at the injection site
- Headache
- Fever
- Nausea and vomiting
- Dizziness
- Fainting may occur after receiving the injection, which may result in falling with injury. Shaking, stiffening, and other seizure-like activity have also been reported.
- Tiredness

- Chills
- Cellulitis- a non-contagious bacterial skin infection around the injection site

### Blood drawing

Taking blood may cause some discomfort, lightheadedness, bleeding, swelling, or bruising where the needle enters the body, and in rare cases, fainting or infection.

### Pap test, cervical brush, anal swab

These tests can be uncomfortable. The discomfort will stop as soon as the test is done. Occasionally there can be some slight bleeding from the cervical brush.

### Oral exam

The oral exam can be uncomfortable, but is usually painless.

## **RISKS RELATED TO PREGNANCY**

Condoms are always highly recommended for HIV-infected women, to decrease the chance of passing HIV to a partner.

The vaccine in this study may be unsafe for unborn babies. If you are having sex that could lead to pregnancy, you must agree not to become pregnant.

Because of the risk involved, you and your partner must use at least one method of birth control that you discuss with the study staff. You must continue to use at least one method until 1 year after the last vaccination. You may choose one or more of the birth control methods listed below:

- Condoms (male or female) with or without a spermicidal agent
- Diaphragm or cervical cap with spermicide
- IUD
- Hormone-based contraception

If you are taking certain anti-HIV drugs (Efavirenz), you and your partner must use at least two of these methods of birth control. The study staff will discuss this with you.

If you think you may be pregnant at any time during the study, tell your study staff right away. The study staff will talk to you about your choices.

If you become pregnant during the study, you will not receive any more vaccinations. If you decide to stay in the study, you will be asked to sign a pregnancy consent form. If you agree, you will continue to have most of the study tests. However, you will not have the pelvic exam, anal swab or Pap smear done.

Also, study staff will contact you to ask you about the outcome of the pregnancy.

## **ALTERNATIVES TO TAKING PART IN THIS STUDY**

Your medical care will not be affected by whether or not you choose to take part in this study. You can choose to receive the HPV vaccine without being in this study at all. Please talk to your provider about these options.

## **BENEFITS OF THE STUDY**

If you take part in this study, there may be a direct benefit to you, but no guarantee can be made. It is also possible that you may receive no benefit from being in this study. Information learned from this study may help other HIV-positive women.

### **MEDICAL RECORD INFORMATION**

We will include study information in your \_\_\_\_\_ medical record. If you do not have an \_\_\_\_\_ medical record, one will be created for you even if your only connection with \_\_\_\_\_ is as a research subject.

#### **Information that will be put into your medical record**

Your name, address, telephone number, date of birth, social security number, health insurance information, billing information, and any other information you provide on the hospital or clinic information form.

#### **Who will have access**

This medical record will be permanent. It will be stored with all other \_\_\_\_\_ medical records. A copy of your record may also be stored on the secure \_\_\_\_\_ medical record computer system. Access to medical records and the computer system is restricted to only authorized staff with passwords for the system. Only \_\_\_\_\_ staff and people who have legal access to your medical record will be able to see it. This may include your insurance company and government regulatory agencies. If you have already given permission to anyone (such as your health insurance company) to look at your medical record, they may receive this research information if they ask for a copy of your medical record.

### **OTHER INFORMATION**

#### **Prohibited Medications**

If you are not taking any immune modulating herbal supplements/immune enhancers at study entry, you cannot start taking them during the duration of the study.

#### **Precautionary Medications**

No standard of care vaccinations during the 2 weeks preceding viral load measurements.

#### **Test and study results**

We will tell you the results of the following tests as soon as this information becomes available: routine blood safety tests, HIV viral load, CD4+/CD8+ cell counts, pregnancy tests, and Pap smears.

We will not tell you the results of the HPV tests from the cervix, anus or mouth, or the HPV antibody test results. We will also not tell you the HIV viral load from the cervix. All of these tests will be done at some time in the future.

We will tell you about new information from this or other studies that may affect your health, welfare, or willingness to stay in this study. At the end of the study, we will tell you when study results may be available and how to learn about them.

#### **Compensation for Injury**

If you think you have an injury or illness related to this study, contact the study staff listed at the top of the consent form right away. They will treat you or refer you for treatment. The \_\_\_\_\_ will pay up to \$\_\_\_\_\_ to reimburse for treatment of injury or illness resulting from the study. No money has been set

aside to pay for things like lost wages, lost time, or pain. However, you do not waive any rights by signing this consent form.

### **Costs of the study**

You and your insurance will not be billed for study-related visits, study vaccinations, physical examinations, laboratory tests, or other procedures.

You will receive \$\_\_\_\_ per visit (for most visits) for participating in this study. You will receive \$\_\_\_\_ for the entry visit, week 28 and week 52 visits where you will have a pelvic, anal and possible oral swab/exam. You will receive a total of \$\_\_\_\_\_ if you complete all of the study visits. If you cannot make a visit due to transportation or childcare issues, there are additional funds to help offset those costs and you should contact your study clinician.

### **Stopping the study**

The study doctor may need to take you off the study early without your permission if:

- The study is cancelled by the U.S. Food and Drug Administration (FDA), the National Institutes of Health (NIH), the Office for Human Research Protections (OHRP), other government agencies, the ACTG, Merck & Co, Inc (the drug company providing the HPV vaccine), or the UW Institutional Review Board (IRB: committee that watches over the safety and rights of research participants.)
- A Study Monitoring Committee recommends that the study be stopped early
- You are not able to attend the study visits
- Your doctor no longer thinks participating in the study is in your best interest
- You did not receive the vaccine

The study doctor may also need to take you off the study vaccine without your permission if:

- Continuing the study vaccine may be harmful to you
- You need a treatment that you may not take while on the study
- You become pregnant or begin breast-feeding
- You are not able to keep up with the vaccine schedule

If you must stop having the study vaccine before the study is over, we may ask you to continue to be part of the study and return for all study visits and procedures.

### **Confidentiality**

Although we will make every effort to keep your information confidential, no system for protecting your confidentiality can be completely secure. It is possible that unauthorized persons might discover that you are in this study, or might obtain information about you. University and government oversight offices such as the ACTG, the U.S. Food and Drug Administration (FDA), \_\_\_\_\_ Regulatory Offices, the National Institutes of Health (NIH), the Federal Office for Human Research Protections (OHRP), study staff, and study monitors, sometimes review studies such as this one to make sure they are being done safely and legally. If a review of this study takes place, your records may be examined. The reviewers will protect your privacy. The study records will not be used to put you at legal risk of harm. We will not release any information that identifies you without your written permission, except as described below.

We will ask you to sign a medical release of records form. This will allow us to obtain information from your primary care provider and to send study information, like your test results, to that provider using our hospital's electronic medical records system. We will need to have access to your medical records for this study. For example, we may need to look at records of previous illnesses and blood tests or records about illnesses that occur during the study. If you are not eligible for the study after completion of all screening tests, your results will be kept indefinitely in a confidential file at the ACTU.

To help us protect your privacy, we have obtained a Certificate of Confidentiality from the Federal Government. With this Certificate, we cannot be forced to disclose information in our research records that may identify you, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. However, there are some limits to the Certificate. It cannot be used to resist a demand for information from authorized federal employees, when they want the information to (1) audit or evaluate a federally-funded research project, or (2) meet the requirements of the federal Food and Drug Administration.

The Certificate does not prevent us from voluntarily disclosing identifiable information. We will make the following voluntary disclosures of identifiable information, which may include your name. Any of this information that is disclosed will not be protected by the Certificate of Confidentiality.

- To the study sponsor
- To state or federal public health authorities to whom certain contagious diseases (tuberculosis, HIV, anthrax, syphilis) are reported (if we observe such diseases in any subjects) In the State of \_\_\_\_\_, researchers are not required to report HIV infection to the health department, but we are required to remind your personal physician that HIV is a reportable disease.
- To law enforcement authorities: information that suggests the occurrence of child abuse, elder abuse, or your intent to immediately and substantially harm yourself or others.
- To your \_\_\_\_\_ medical record: specific information as described elsewhere in this form
- To state, federal, and institutional offices involved in auditing or compliance of research, risk management, participant safety, or financial controls.

In addition, any study test or procedure results or other study information or documents [including this consent form] that are included in your medical record will not be covered by the Certificate of Confidentiality and may be released if requested by a lawful subpoena or other lawful and appropriate request for the information.

Persons who have access to your medical record will have access to any research-related information or documents that are in your record. Access to your medical records is governed by \_\_\_\_\_ state law (RCW \_\_\_\_\_) and by the federal HIPAA law.

The Certificate of Confidentiality does **not** prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. If you give your written consent to an insurer, employer, or other person to receive research information about you, then we can not use the Certificate to withhold that information. The Certificate of Confidentiality is not an endorsement from the Federal Government for our research.

Your study information will be sent to a database in \_\_\_\_\_, and will be identified only by a code. The database contains all study information, such as subject characteristics, study drugs, and



Storage of leftover samples is not necessary to participate in the study. Even if you agree now you may withdraw your approval for the storage of your leftover samples at anytime in the future. Please indicate your choice below WITH YOUR INITIALS:

\_\_\_\_\_ YES      \_\_\_\_\_ NO

Copies to:      Subject  
                    Subject's Research File