WOMEN'S INTERAGENCY HIV STUDY
SECTION 19: AIDS AND CANCER SPECIMEN RESOURCE (ACSR) AND PATHOLOGY REVIEW PROTOCOLS

I. ACSR PROTOCOL FOR GENITAL BIOPSY COLLECTION

A. OBJECTIVE

To collect specimens for ACSR donation from 10 to 20 women at each site who have clinically indicated colposcopies.

B. PARTICIPANT ELIGIBILITY AND ENROLLMENT

All WIHS participants (HIV-infected and HIV-uninfected) who have indication for a colposcopy according to WIHS protocol are eligible to participate. A lesion need not be found during colposcopy for a participant to be eligible for enrollment. A target number of 10 to 20 women per visit are recommended for enrollment at each WIHS site.

All participants who meet the eligibility criteria will be asked to sign an ACSR consent form prior to the colposcopy, phlebotomy and oral rinse collection.

C. BIOPSY PROCEDURES

1. BIOPSY THE LESION

   If the biopsy is bigger than 0.4 cm, split the lesion in half. One half should be put in formalin as per WIHS colposcopy protocol and the other half should be snap frozen. If the biopsy is smaller than 0.4 cm, put in formalin as per WIHS colposcopy protocol and attempt to take a second biopsy from the same lesion or another lesion.

2. BIOPSY A NON-LESIONAL SITE

   This is the control specimen and should be taken whether or not there is sufficient lesional biopsy material for the ACSR. When possible, the non-lesional biopsy should be obtained from the side of the cervix contralateral to the lesion, in a similar region as the lesion. However, if upon colposcopic examination it is discovered that there is no lesion, the control specimen should still be collected for donation. The specimen can be collected from any location on the cervix in this case.

D. TISSUE HANDLING PROCEDURES

1. If you plan to perform more than one biopsy in one day you should write the WIHSID number and indicate “lesion” or “control” on the disposable tissue block. You can write with a black Sharpie™ or a wax pencil.

2. After collecting your biopsy do the following:

   a. Place the biopsy on a piece of gauze.

   b. Transfer the biopsy from the gauze with your forceps.

   c. Place the biopsy into the depression of the tissue mounting block epithelium or connective tissue side down. In other words, place the tissue sample flat onto the block.

   d. Pour OCT over the tissue until you fill the depression of the block.

   e. Pick up the block containing the embedded tissue with your long forceps.

3. Place the block into the thermos and hold it until the OCT block stops sizzling. If you plan to clean up first or perform another biopsy just leave the blocks in the thermos until you are ready to transfer the blocks into the Nunc vials. To eliminate confusion, it is advisable not to store more than one participant's biopsies in the thermos.

4. Label the Nunc vials with the WIHSID labels (must be able to stick after being in liquid nitrogen) or you can write with a pencil on the white writing space of the Nunc vial and indicate whether it is lesion or non-lesion (control tissue) on the vial.

5. Pop the tiny block of OCT embedded tissue from the disposable tissue block and place it into the Nunc vial.

6. Prepare the ***ACSR Specimen Submission/Pathology Data Form*** (see **Appendix A** to this section) corresponding to the appropriate WIHSID. In the #3 "Note" field, indicate the WIHS visit date just prior to the date the specimen was obtained, except when the specimen was obtained on the same date as the WIHS visit.

7. FedEx on dry ice pellets (available through Pathology Departments) to the appropriate ACSR Site (see **Section G**).

E. BLOOD COLLECTION PROTOCOL

Important: *Blood should only be collected if there was ACSR biopsy material collected, separated and frozen.*

For women with ACSR biopsy material, one 10 ml yellow-top tube should be filled with blood and labeled with the WIHSID number and date. The yellow-top tube must be received by the ACSR bank within 24 hours of being drawn so that cells can be separated.

Alternatively, the blood may be separated locally before shipment, using the following procedures:

1. Separate the PBMCs and freeze with DMSO at a concentration of 10 million cells per ml and aliquot 1 ml per Nunc vial.

2. Freeze 1 ml aliquots of the plasma in Starstedt vials.

3. All vials must be labeled with the WIHSID and specimen type. Leave room in the white writing space for the ACSR accession number to be added later by the Bank.

F. ORAL RINSE COLLECTION PROTOCOL

Important: *An oral rinse specimen should only be collected if there was ACSR biopsy material collected, separated and frozen.*

Each participant will be asked to vigorously swish 10 ml of Scope mouthwash or saline in her mouth for 15 seconds, gargle for 15 seconds, and then expectorate into a specimen collection cup labeled with the WIHSID number and date. Collection of the oral rinse sample will take approximately 2 to 4 minutes per participant. Samples should be placed on ice or at 4°C immediately after collection and stored at -20°C until shipment to the ACSR.

Oral Rinse Collection Procedures:

1. Materials needed: Scope mouthwash or saline stock bottle; specimen collection cup; gloves; pen; medicine cup; 50 mL conical centrifuge tube.

2. Put gloves on.

3. Label specimen collection cup with WIHSID number and date.

4. Pour 10 mL of Scope mouthwash or saline into a medicine cup, making sure not to touch the rim of the cup.
5. Hand participant the cup with the mouthwash or saline and the specimen collection cup (with top removed).
6. Time participant while she swishes the mouthwash or saline for 15 seconds in her mouth.
7. Tell the participant when it is time to gargle the mouthwash or saline. If she cannot gargle for 15 seconds, instead have her gargle for 5 seconds, swish the mouthwash or saline in her mouth for 5 seconds, and then gargle again for another 5 seconds.
8. Have the participant spit the mouthwash or saline into the specimen collection cup when done gargling.
9. Take the specimen collection cup from the participant, and, being careful not to touch the rim, screw the top on tightly, and place on ice.
10. Label a 50 mL conical centrifuge tube with the WIHSID, date and "OR."
11. Transfer the oral rinse sample from the specimen collection cup to the 50 mL conical tube.
12. Store the 50 mL conical centrifuge tube at -20°C until ready to ship.
13. Collected oral rinse samples should be shipped to the local ACSR as described in **Section G**.

G. SPECIMEN SHIPMENT INFORMATION

A completed ***ACSR Specimen Submission/Pathology Data Form*** should accompany each specimen submitted to the Bank. **Biopsy, oral rinse and blood specimens** from the Los Angeles, San Francisco and Chicago sites should be sent to the SFGH AIDS and Cancer Specimen Resource (ACSR). The SFGH ACSR address is the following:

Leanne C. Huysentruyt, Ph.D.
Project Manager
AIDS and Cancer Specimen Resource / ACSR
McGrath Labs
University of California San Francisco
1001 Potrero Avenue
Bldg 100, Room 333E
San Francisco, CA 94110
Email: Leanne.huysentruyt@ucsf.edu
Tel: 415-206-5510
Fax: 415-206-6625

In case of problems, call Leanne Huysentruyt at (415) 206-5510, Ron Honrada at 415-206-5434, Alanna Morris at 415-206-5434, or Melissa Ancheta at (415) 206-3858.

All specimens from the Bronx, Brooklyn and Washington, D.C., sites should be sent to the George Washington University Medical Center AIDS and Cancer Specimen Resource. The GWUMC ACSR address is the following:

AIDS and Cancer Specimen Resource
Department of Pathology
George Washington University Medical Center
2300 I Street, NW
Washington, DC 20037 Phone: (202) 994-0434 or (202) 994-3422

Prior to shipping specimens to GWUMC, please call the Bank at (202) 994-0434 or (202) 944-3422 to inform them of the pending shipment and tracking number.

H. ACSR DATA MANAGEMENT

1. Each specimen will be logged in the ACSR database using the WIHSID as the accession number.

2. ACSR staff will e-mail the Project Director of the site to notify him/her of the placement of the specimen in the Bank.

3. On a semi-annual basis, the ACSR will send an electronic list of WIHSIDs for which they have recently received biopsy specimens and the visit number indicated in the #3 "Note" field on the ACSR Specimen Submission/Pathology Data Form. WDMAC will provide the predetermined set of clinical information that is available in the WIHS database to the ACSR. This effort will replace sites having to complete the NCI HIV-Related Malignancies Patient Clinical Data Form. The data will be sent to the ACSR in an electronic data file formatted as an ASCII file. A codebook will accompany the data set so that the delineation of the data is clear to the ACSR. The data that will be sent to ACSR includes, and is limited to, the following data:

   WIHS ID, participant date of birth, date of biopsy read, date of last participant contact, baseline HCV+ status, baseline HTLV 1 & 2 status, baseline CDC risk group, baseline HBV+ status, HIV status, viral load (including limit of test), race, biopsy result (diagnosis), biopsy site, CD4+ count, CD4+ date, CD8+ count, CD8+ date, antiretroviral therapy use since last visit, radiation therapy use since last visit, chemotherapy use since last visit, BV status, oral contraceptive use since last visit, pregnancy status, death status, date of death if applicable, and HPV status at WIHS Visits 1 & 2.

I. SUPPLIES AND MATERIALS

The additional ACSR supplies and materials (as noted below) should be available at the clinic before the procedure.

1. CLERICAL SUPPLIES NEEDED

   - ACSR consent form
   - ***ACSR Ascertainment Tracking Checklist*** (ACSR ATC)
   - ***ACSR Specimen Submission/Pathology Data Form***
   - ***Quality Control Sheet for Review of Gynecologic Material*** (QCGY)
   - Specimen labels (that will stick on frozen tubes) with WIHSID number
   - Specimen labels (that will stick on frozen tubes) with "lesion specimen" or "control specimen"
   - Nalgene™ Cryoware Markers or Sharpie™ Ultrafine Markers

2. MEDICAL SUPPLIES NEEDED

- 10 ml yellow-top tube (need to specify type or CAT# – Fisher 0268426)
- Forceps – tissue
- Long forceps
- Gauze
- OCT tissue mounting media (VWR cat# 25608930)
- Disposable Tissue Embedding Mold – Baxter Scientific part #M7307-1 (7mmx7mm) (Fisher Cat # 15182501A – size 7mmx7mmx5mm)
- Liquid nitrogen
- Liquid nitrogen thermos
- Nunc vials (Fisher Cat # 12565167N)
- Dry ice and containers for specimen transport to lab

II. ACSR PROTOCOL FOR RETRIEVAL OF SELF-REPORTED BIOPSIES FOR DONATION AND REVIEW

A. OBJECTIVES

To collect tissue specimens from various malignancies and other tissue derived from a biopsy or other surgical procedures, which were performed as part of routine patient care and which may occur in WIHS participants. Female-specific tissue specimens are given priority, although all tumor specimens are to be collected where possible.

To review and confirm the diagnoses made from tissue obtained at a WIHS colposcopy by a variety of pathologists in the local community.

Central pathology review of the tissue will be performed in a time frame dependent upon the current academic projects ongoing within the WIHS. The purpose of central review will be to verify cancer diagnoses, optimize the diagnoses and search for unusual histopathology.

B. PARTICIPANT ELIGIBILITY AND ENROLLMENT

All WIHS participants (HIV-infected and HIV-uninfected) who have a clinical indication for a biopsy of a suspected tumor or malignancy are eligible to participate, if the biopsy or procedure was performed. In addition, all patients with history of a tumor or malignancy that is currently under study by the Cancer/Pathology Working Group of the WIHS will be targeted for enrollment in this ACSR protocol. Female-specific tissues collected as part of the WIHS visit are not collected for this part of the protocol. A target number of four to five women per visit number is recommended for enrollment at each WIHS site.

All participants who meet the eligibility criteria will be asked to sign an ACSR consent form and release form, permitting access to the biopsy material from the institution where the biopsy was performed.

C. VERIFICATION OF SELF REPORT AND PATHOLOGIC DIAGNOSIS

1. The interviewer will ascertain that the participant has undergone a biopsy at any time since the last study visit and ask the participant for permission to collect a portion of the material for submission to the ACSR. If the participant refuses to donate her tissue, the interviewer will write "REFUSED DONATION" on the ***ACSR ATC*** form. When this happens, the interviewer will ask the participant for permission for a WIHS pathologist to review her diagnostic slides and then return them. If the participant agrees, the interviewer will obtain consent for review and write "REFUSED DONATION BUT REVIEW PERMITTED" on the ***ACSR ATC*** form. If the participant refuses permission for both, the interviewer will write "REFUSED DONATION AND REVIEW" on the ***ACSR ATC*** form, and no further action will be taken.

2. The interviewer will collect information indicating the details (from what site the biopsy was taken, the date of the biopsy and the institution/clinic where the biopsy or procedure occurred) on the ***ACSR ATC*** form and obtain consent for review of the participant's medical records and consent for donation of the tissue to the ACSR.

3. The ***ACSR ATC*** form will be given to the ACSR abstractor. The original form should be filed with the rest of the visit forms and a copy given to the abstractor. The ACSR abstractor reviews the ***ACSR ATC*** form and gives priority to female-specific tissue specimens (i.e., breast, vulva, vagina, cervix, uterus, ovaries and anus) for abstraction, especially those tissues obtained at colposcopies performed by non-WIHS clinicians. To facilitate the collection of tissues from non-WIHS colposcopies, a prompt has been added to form ***L14*** for clinicians to complete the ***ACSR ATC*** whenever a participant receives a non-WIHS colposcopy.

   In the future, if the WIHS EC, after consultation with the Cancer/Pathology Working Group wants to expand beyond female-specific tissue and include more tissue sites, they need only inform the ACSR abstractor to obtain these reports also.

4. The abstractor (designated ACSR staff or Outcomes Ascertainment abstractor) will obtain a copy of the pathology report or transcribe the report onto the old MRA form ***CA4***, currently used as a "worksheet" at some sites.

5. Pathology reports obtained from medical record abstraction will be reviewed by the site-specific, NCI-sponsored ACSR-designated pathologist.

6. The NCI-sponsored ACSR-designated pathologist at each site will determine which tissues should be obtained for review, and will inform the appropriate individual (abstractor or other) at each site. Each site will determine the interval at which these self reports will be evaluated. With consent, the pathologist (or his/her designee) will review all specimens obtained at a non-WIHS colposcopy and document his/her findings on the ***Quality Control Sheet for Review of Gynecologic Material*** (QCGY).

7. Central pathology review will be performed on all cancer diagnoses confirmed by medical record abstraction or Cancer Registry match. If the participant agrees to donation and review:

   a. Request the tissue block from the institution holding the specimen, to be processed as described in **Section II.D**, below.

   b. Additionally, request all of the slides made from the biopsy, or at a minimum, the diagnostic slide(s) upon which the original diagnosis was made.

   c. The local WIHS pathologist will review this material and decide which slides to send for central review. Prior to shipping specimens to GWUMC, call (202) 994-0434 or (202) 944-3422 to inform them of the pending shipment and tracking number. Note that this is a slightly different address than that of the ACSR at GWU. Ship to:

      Jan Orenstein, M.D.
      Department of Pathology
      Ross 502
      George Washington University Medical Center
      2300 I Street, NW
      Washington, DC 20037

   d. Dr. Orenstein and/or his designated pathologists (depending on the type of tissue and their areas of expertise) will review the slides and fill out the ***Quality Control Sheet for Central Review of Surgical Specimens*** (QCSS), outlining the results of the central review.

   e. Data entry of the ***QCSS Form*** will be accomplished at the Georgetown WIHS site.

If the participant agrees only to a review of the cancer diagnosis, follow the procedures outlined in **Section II.C**, item 7, steps b–c, above.

D. SPECIFIC PROCESS FOR OBTAINING THE CLINICALLY-DERIVED TISSUE SPECIMENS

1. Designated ACSR staff will send the participant's signed ACSR consent form with a request to the institution where the biopsy was taken, asking for the following:
   a. Block of representative tissue, OR
   b. 10 unstained slides from the block.
   c. If 10 unstained slides cannot be released, we will take whatever is possible within this range.
   d. Each section should be 4μM in thickness.
   e. If the institution agrees to send the block instead of slides, 10 unstained slides, each 4μM in thickness, will be made by the ACSR pathologist at each site after the block has been received. In addition, a 30μM section will be placed in a Nunc or microfuge tube for potential use in PCR. The block will then be returned to the institution from which it came.
2. Designated ACSR staff will follow-up with the institution in question to assure that the material is sent to the WIHS site. ACSR staff will also receive the tissue.
3. Designated ACSR staff will label all slides with (1) WIHSID and (2) designation of "control" versus "lesion" status. (Presumably all such tissues will be "lesion.")
4. Prepare the ***ACSR Specimen Submission/Pathology Data Form*** corresponding to the appropriate WIHSID. In the #3 "Note" field, indicate the WIHS visit date just prior to the date the specimen was obtained, except when the specimen was obtained on the same date as the WIHS visit.
5. Designated ACSR staff will ship the unstained, labeled slides with the completed ***ACSR Specimen Submission/Pathology Data Form*** to the ACSR.

E. SPECIMEN SHIPMENT INFORMATION

The ***ACSR Specimen Submission/Pathology Data From*** should accompany each specimen submitted to the Bank. Specimens from the Los Angeles, San Francisco and Chicago sites should be sent to the SFGH Specimen Resource. The SFGH ACSR address is the following:

Leanne C. Huysentruyt, Ph.D.
Project Manager
AIDS and Cancer Specimen Resource / ACSR
McGrath Labs
University of California San Francisco
1001 Potrero Avenue
Bldg 100, Room 333E
San Francisco, CA 94110
Email: Leanne.huysentruyt@ucsf.edu
Tel: 415-206-5510
Fax: 415-206-6625

In case of problems, call Leanne Huysentruyt at (415) 206-5510, Ron Honrada at 415-206-5434, Alanna Morris at 415-206-5434, or Melissa Ancheta at (415) 206-3858.

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F. ACSR DATA MANAGEMENT

1. Each specimen will be logged in the ACSR database using the WIHSID as the accession number.

2. ACSR staff will e-mail the Project Director of the site to notify him/her of the placement of the specimen in the Bank.

3. On a semi-annual basis, the ACSR will send an electronic list of WIHSIDs for which they have recently received biopsy specimens and the visit number indicated in the #3 "Note" field on the ***ACSR Specimen Submission/Pathology Data Form***. WDMAC will provide the predetermined set of clinical information that is available in the WIHS database to the ACSR. This effort will replace sites having to complete the NCI HIV-Related Malignancies Patient Clinical Data Form. The data will be sent to the ACSR in an electronic data file formatted as an ASCII file. A codebook will accompany the data set so that the delineation of the data is clear to the ACSR. The data that will be sent to ACSR includes, and is limited to, the following data:

   WIHS ID, participant date of birth, date of biopsy read, date of last participant contact, baseline HCV+ status, baseline HTLV 1 & 2 status, baseline CDC risk group, baseline HBV+ status, HIV status, viral load (including limit of test), race, biopsy result (diagnosis), biopsy site, CD4+ count, CD4+ date, CD8+ count, CD8+ date, anti-retroviral therapy use since last visit, radiation therapy use since last visit, chemotherapy use since last visit, BV status, oral contraceptive use since last visit, pregnancy status, death status, date of death if applicable, and HPV status at WIHS Visits 1 & 2.

G. SUPPLIES AND MATERIALS

The additional ACSR supplies and materials (as noted below) will be required:

1. CLERICAL SUPPLIES

   - ACSR consent form
   - ***ACSR Ascertainment Tracking Checklist*** (ACSR ATC)
   - ***ACSR Specimen Submission/Pathology Data Form***
   - ***Quality Control Sheet for Review of Gynecologic Material*** (QCGY)
   - ***Quality Control Sheet for Central Review of Surgical Specimens*** (QCSS) (needed by Dr. Jan Orenstein and/or designated pathologists at GWUMC only)
   - Specimen labels (that will stick to slides) with WIHSID number
   - Specimen labels (that will stick to slides) with "lesions specimen" or "control specimen"
   - Small slide shipping box
   - Shipping label

III. REVIEW OF BIOPSIES OBTAINED AT WIHS COLPOSCOPY

A. OBJECTIVE

Review and confirmation of diagnoses made of specimens obtained at WIHS colposcopies will ensure consistency and quality assurance.

B. PARTICIPANT ELIGIBILITY

Participants who have had colposcopy performed by a WIHS clinician and who have given consent for review. The local WIHS pathologist will determine which cases will be reviewed.

C. VERIFICATION OF PATHOLOGIC DIAGNOSIS

1. Designated staff will send the participant's consent with a request to the institution where the biopsy was taken, asking for all of the slides made from the biopsy, or at a minimum, the diagnostic slide(s) upon which the original diagnosis was made.
2. The local designated WIHS site pathologist will review the slides and record his/her findings on the ***Quality Control Sheet for Review of Gynecologic Material*** (QCGY).
3. Slides will be returned to the institution from which they were borrowed.

D. SUPPLIES AND MATERIALS

The additional supplies and materials (as noted below) will be required:

1. CLERICAL SUPPLIES
    - Consent form
    - ***Quality Control Sheet for Review of Gynecologic Material*** (QCGY)

APPENDIX A: AIDS AND CANCER SPECIMEN RESOURCE SPECIMEN SUBMISSION/PATHOLOGY DATA FORM