WOMEN'S INTERAGENCY HIV STUDY

SECTION 9: MEDICAL EXAM

The WIHS medical exam is to be performed as noted in the **Manual of Operations, Section 7**. The medical exam should preferentially be performed at the end of the core interview, or before the Neurocognitive (NC) Battery during visits when the NC Battery is being administered. The medical exam may also be performed after administration of follow-up interview forms *F21* through *F23*. It is unacceptable to perform the medical exam prior to the administration of interview forms *F21* through *F23* except in rare situations, or when the NC Battery is being administered. Refer to **Section 7, Follow-Up Visits, Section E,** for a detailed explanation of preferred and acceptable visit completion order.

The medical exam consists of:

I. Arterial Brachial Index (ABI) Measurement **(see MOO, Section 38, for protocol)**
II. Neuropathy assessment
III. General physical exam
III. Urine collection (for pregnancy testing and repository, when applicable)
IV. Gynecological exam
V. Colposcopy (if indicated)
Biopsy (if needed)
Treatment (as indicated)

It is preferable to perform all components of the medical exam on the same day, with ABI performed prior to phlebotomy. Results from the medical exam should be recorded on the following forms: *Arterial Brachial Index Measurement Form (ABI), Baseline/Follow-up Neuropathy Signs and Symptoms (NP01/02), Physical Exam (F7r/F07), Gynecological Exam (F08)* and, if indicated, *Colposcopy (L14), Biopsy (L15), Dysplasia Treatment (L16),* and *Colposcopy Tracking (COLPO)* forms. Forms *NP01/02, F07* and *F08* have been designed to reflect the sequence in which the exams are to be performed. Following is a detailed description of what each exam component entails.

I. NEUROPATHY ASSESSMENT

Baseline neuropathy signs and symptoms (*NP01*) should be evaluated at visit 27 for **1994/95 and 2001/02 recruits**. If a participant misses visit 27, then the *NP01* should be completed at visit 28. **2011/12 recruits** will receive their baseline neuropathy evaluation at the enrollment visit. **WIHS-V recruits** will receive their baseline neuropathy evaluation at the first follow-up visit. Follow-up neuropathy assessments (*NP02*) will begin during visit 30, and will be conducted annually thereafter at all **even-numbered follow-up** visits for all recruits.

The neuropathy evaluation consists of questions about the type of neuropathy symptoms that participants may experience, an evaluation of the participant's perception of vibration using a Rydel-Seiffer 64/128 Hz tuning fork, and an evaluation of the participant's ankle and knee deep tendon reflexes using a Queen's Square hammer. Knee deep tendon reflexes were not evaluated at baseline for 1994/95, 2001/02 and 2011/12 recruits, but this assessment should be performed for the WIHS-V baseline and follow-up assessments. The participant need only remove her shoes and socks for this part of the exam. Specific instructions for the neuropathy assessment are described in the *NP01* and *NP02* Question-by-Question Specifications (QxQs).

NOTE: DO NOT perform neuropathy evaluation unless participant has two legs and two feet.

Each WIHS site will designate a physician (usually a neurologist, but not required to be a neurologist) to be responsible for training and certification of examiners. The tester and the training physician must agree on at least eight out of 10 assessments for the tester to be certified. At each site in a clinic with a high

prevalence of neuropathy, the tester will perform reflex assessment followed by the trainer assessing reflexes on the same subject.

II. GENERAL PHYSICAL EXAM

A. HEIGHT and WEIGHT

Height should be measured at baseline, visit 9, at every visit from visit 16 through visit 20, and at even-numbered visits only beginning with visit 22. Participants should be weighed at every visit, with clothes and shoes off, in underwear or gown.

1. WEIGHT

   MATERIALS NEEDED: Balance scale

   PROCEDURES: A balance scale should be used, and all weights should be recorded in pounds (LBS). The scale should be level and on a firm surface (not a carpet). Be sure the scale is balanced so that the indicator is at zero when no weight is on the scale. The participant should be instructed to stand in the middle of the platform of the balance scale with head erect and eyes looking straight ahead. Adjust the weight on the indicator until it is balanced. The weight should be recorded in pounds to the nearest 1.0 lb. Please do not make any conversions from kilograms.

   Have the participant step off the scale, reset the balance to zero and repeat. If measures differ by more than 1.0 lb., repeat a third time. Always record the first measure that most closely matches the last measure. For example, if only two measures were taken (i.e., the first and second measures were within 1.0 lb. of one another) record the first measure taken. If three measures were taken and the second and the third are within 1.0 lb. of each other, record the second measure. If three measures were taken and the first and the third measure were within 1.0 lb. of each other, record the first measure.

2. HEIGHT

   PROCEDURES: Ask the participant to remove any hair ornaments, jewelry, buns, or braids from the top of the head. Have the participant stand up straight with the body weight evenly distributed and both feet flat on the scale platform. Instruct the participant to stand with the heels together and toes apart. The toes should point slightly outward at approximately a 60° angle. Check that the back of the head, shoulder blades, buttocks, and heels make contact with the backboard.

   Second, align the head in the Frankfort horizontal plane. The head is in the Frankfort plane when the horizontal line from the ear canal to the lower border of the orbit of the eye is parallel to the floor and perpendicular to the vertical backboard. Many people will assume this position naturally, but for some participants the examiner may need to gently tilt the head up or down to achieve the proper alignment. Instruct the participant to look straight ahead.

   Next, lower the head piece so that it rests firmly on top of the participant's head, with sufficient pressure to compress the hair. Instruct the participant to stand as tall as possible, take a deep breath, and hold this position. The act of taking a deep breath helps straighten the spine to yield a more consistent and reproducible stature measurement. Notice that the inhalation will cause the head piece to rise slightly.

   Some participant's have hair styles such as a barrette, bun, or braid that will interfere with the placement of the stadiometer head piece. Other participants may refuse to remove their shoes for the height measurement. In these cases, the examiner should enter "-9" (data missing) for the participant's height. **NOTE: Measurement of height at the baseline visit is mandatory.**

B. BODY HABITUS

In this portion of the exam, the circumferences of the participant's upper arm, breasts, waist, thigh and hips are to be measured. In addition, the exam will include assessment of the participant's dorsocervical fat pad, if applicable. Body habitus measurements should not be performed on pregnant women.

NOTE: All measurements should be taken at least two times. If the difference between the first two measures exceeds 0.7 cm, repeat the measure a third (and final) time.

MATERIALS NEEDED: Cloth or disposable paper measuring tape

PROCEDURES: Body measurements should always be taken on the right side of the body (unless for a specific reason such as casts or amputations). Any marks that need to be made on the participant's skin should be made with a cosmetic pencil (waxed base) such as an eyeliner pencil. The measuring tape should be flexible but non-stretchable (i.e., Gulik type) and measurements should be recorded to the nearest 0.1 cm.

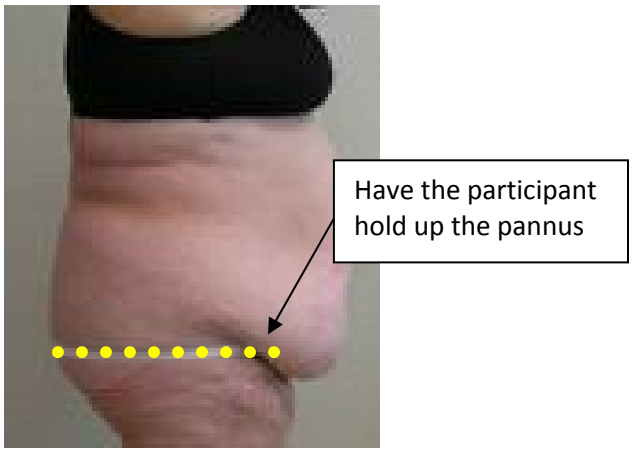
NOTE: If a body measure is above the measurable limits of the tape (i.e., 180+), "999" should be entered into the recording space for that measure.

1) Upper Arm Girth: Have the participant stand erect with feet together and the right arm flexed 90° at the elbow with the palm facing up. The examiner should be positioned behind the participant. Using a tape measure, mark a point halfway between the lateral projection of the acromian process of the scapula (bump on the backside of shoulder) and the interior part of the olecranon process (elbow). Next, the participant should stand with the right elbow relaxed so that the right arm hangs freely to the side. The examiner should stand facing the participant's right side. The measuring tape should be placed around the upper arm at the marked point perpendicular to the long axis of the upper arm. The tape should be held so that the zero end is held below the measurement value. The tape should rest on the skin surface, but not be pulled tight enough to compress the skin. The arm circumference will be recorded to the nearest 0.1 cm.

2) Chest Girth: The chest girth should be measured at the level of the fourth costo-sternal joints, which laterally corresponds to the level of the sixth ribs. The fourth costo-sternal joint can be located by a two-handed palpitation method whereby the examiner places both the index fingers on the superior surfaces of the clavicles, while the thumbs locate the first intercostal space. The index fingers then replace the thumbs, which are lowered to the second intercostal space. This procedure can then be repeated until the fourth costo-sternal joint is located. The ribs and their costal cartilages are followed medially to their articulations at the sternum, and this point should be marked. The participant should be standing with the feet at shoulder width. The arms should be slightly away from the body to allow placing the tape around the chest. The measuring tape should be placed horizontally at the marked point. Once the tape is in place, the arms can be lowered to their relaxed position. Take the measurement at the end of a normal expiration. The chest girth will be recorded to the nearest 0.1 cm.

3) Waist Girth: The study participant should be in a standing position. The participant should be asked to hold up her gown. The examiner should stand behind the participant and palpate the hip area for the right iliac crest (see *F07* QxQs, **Appendix A**). The examiner should mark a horizontal line at the high point of the iliac crest and then cross the line to indicate the midaxillary line of the body. The pants and underclothing of the participant must be lowered slightly for the examiner to palpate directly on the hip area for the iliac crest. The examiner then should stand on the participant's right side and place the measuring tape around the trunk in a horizontal plane at this level marked on the right side of the trunk. Make sure that the tape is parallel to the floor and that the tape is snug, but does not compress the skin. The measurement will be made at minimal respiration to the nearest 0.1 cm.

4) Hip Girth: The study participant should stand erect with feet together and weight evenly distributed on both feet. The participant should be holding up the examination gown. If the participant has folds of abdominal fat (pannus) that interfere with the ability to accurately measure the hip circumference, have participant lift pannus up during measurement. **Do not include pannus in measurement.**The examiner should place the measuring tape around the buttocks. The tape should be placed at the maximum extension of the buttocks (see *F07* QxQs, **Appendix B**). The examiner then should adjust the sides of the tape and check the front and sides so that the plane of the tape is horizontal. The zero end of the tape should be held under the measurement value. The tape should be held snugly but not tight. The examiner should take the measurement from the right side and record to the nearest 0.1 cm. (See figure on next page.)



5) Thigh Girth: First, have the participant sitting with her right knee bent at a 90° angle. Mark the nearest border of the patella (knee cap). A measuring tape should be placed at the superior aspect of the inguinal crease, which is easily located if the hips are in the sitting position. No pressure should be applied at inguinal crease; however, folds of fat tissue may have to be lifted on some obese participants to measure at the crease. The exam gown should be lifted. The tape should be extended along the midline of the thigh to the line just proximal to the patella (see *F07* QxQs, **Appendix C**). The examiner should make a mark (+) at the midpoint of the thigh with a cosmetic marker. Next, have the participant stand with her right leg just in front of her left leg and her weight shifted back from her left leg. This instruction should be demonstrated by the examiner. The edge of the examining table may be used for the participant to hold onto to maintain balance. The examiner should stand on the participant's right side and the measuring tape should be placed around the midthigh at the marked point. The tape should be positioned perpendicular to the long axis of the thigh with the zero end of the tape held below the measurement value. The tape should rest firmly on the skin without compressing the skin. The thigh circumference will be measured to the nearest 0.1 cm.

C. SKINFOLD MEASUREMENT

NOTE: Skinfold Measurements were discontinued at visit 23.

D. BIOELECTRIC IMPEDANCE ANALYSIS

Bioelectric impedance analysis (BIA) should be used to measure the resistance (Rx) and reactance (Xc) of the participant. The measurements should be taken and recorded on *F07*.

MATERIALS NEEDED: Bioelectric impedance analysis machine – RJL, model #BIA-101Q or RJL, model Quantum II.

GENERAL INSTRUCTIONS:

- ***BIA should not be done on pregnant women, on women who are overheated (as indicated by high body temperature), or on women who have a cardiac pacemaker or who have amputations other than fingers or toes.***
- There should be no portable electrical heater or other electronic device in use in the exam room and the exam table should be non-conductive.
- The battery should be kept current and the equipment should be calibrated weekly.

PROCEDURES:

1) The participant should remove her right shoe and sock. If, for some reason, the procedure must be done on the left side, then make note of this in the participant's chart and, on subsequent visits, always use the left side.
2) The participant should lay on her back, without a pillow, on the exam table, with her arm 30 degrees from her body and thighs not touching.
3) Remove jewelry on the electrode sites.
4) The sites where you will place the electrodes should be gently cleaned with an alcohol wipe, particularly if the skin is moist or covered with lotion. Allow alcohol to evaporate before placing electrodes.
5) Attach the electrodes (use whole electrode pads only) and patient cables as described below and as shown in the photographs provided by RJL. Attach the lead wires to the electrodes with the red leads attached to the wrist and ankle and the black leads attached to the hand and foot. In each case, the red alligator clip should be proximal and the black clip distal.
    - Right wrist*:* Draw an imaginary line on the dorsal surface bisecting the styloid processes of the ulna and radius. Place the center of the electrode along the middle of the imaginary line, and with the tab of the electrode facing out (away from the body).
    - Right hand*:* Place the electrode below the knuckle and above the base of the middle finger, with the tab of the electrode facing out.
    - Right ankle*:* Draw an imaginary line on the dorsal surface of the foot bisecting the medial and lateral malleoli of the ankle. Place the center of the electrode along the middle of the imaginary line with the tab of the electrode facing out.
    - Right foot*:* Place the electrode at least four to five centimeters away from the electrode on the ankle, below the base of the second toe, with the tab of the electrode facing out.
6) The participant should remain motionless and relaxed with her arms and legs slightly apart, never touching any other part of the body. The arms should be bent slightly at the elbow with palms down. In cases where the participant's arms and legs cannot be properly spread (because the participant's body is large), the procedure should still be completed and a note made in the comments section on *F07*. As long as there is no skin contact (the paper gown can be used to separate the arms from the trunk or the legs from each other), no interference with the proper flow of the current should take place.

7) Turn on the analyzer and, when the measurements have stabilized, read and record the displayed Resistance (Rx) and Reactance (Xc) in the spaces provided on *F07*. If you were unable to obtain the reading for either of these two measures, enter "-9" for the respective measure.

8) Turn off the analyzer. Double check the leads and electrodes. Stabilize the participant, turn the leads back on, read and record the displayed Resistance and Reactance in the spaces provided on *F07*. Once again, enter "-9" if you are unable to obtain a reading for either of these measures.

9) Unhook the leads and remove and dispose of the electrodes. Do not reuse the electrodes.

Please note on the form if the participant reports recently having diarrhea, having thrown up, being diaphoretic or incontinent, or any other factors that may affect the BIA measurement.

E. SKIN EXAM

NOTE: The Skin Exam was discontinued at visit 23. It was performed at the baseline visit or 2011-12 recruits, but will not be performed at the baseline visit of WIHS V recruits.

F. ORAL EXAM

NOTE: The Oral Exam was discontinued at visit 21. It was performed at the baseline visit or 2011-12 recruits, but will not be performed at the baseline visit of WIHS V recruits.

G. ASSESSMENT OF LYMPH NODES

NOTE: The assessment of lymph nodes was discontinued at visit 17.

H. BREAST EXAM

Perform the breast exam and record your overall assessment on the *F07* or *F7r*. Beginning with visit 21, the breast exam will be completed only at even-numbered visits and at baseline, unless it is clinically indicated that the participant receive the exam every six months.

I. BLOOD PRESSURE MEASUREMENT

The purpose of implementing a specific protocol for the measurement of blood pressure (BP) in the WIHS is to minimize error in measurements and to standardize the blood pressure measurements according to accepted cardiovascular epidemiology techniques. High blood pressure (HBP or hypertension) is one of the major modifiable risk factors for stroke, coronary heart disease, congestive heart failure, renal failure and peripheral vascular disease. With the implementation of the Cardiovascular Substudy within the WIHS at visit 20, it is vital that blood pressure measurement techniques and training be standardized across all WIHS sites and subsites.

All blood pressure measurements in the WIHS will be collected using the same automated Dinamap monitor (Dinamap Procare Series, GE Medical Systems) for standardization purposes. Each site should purchase a sufficient number of Dinamap monitors so that all WIHS participants seen at all subsites will have their blood pressure measured using the Dinamap monitor.

The WIHS requires the collection of three seated blood pressure measurements from the participant's right arm, using an automated Dinamap blood pressure monitor. The pulse rate will be recorded with each blood pressure measurement from the Dinamap monitor. The Dinamap monitor should be set to automatically measure blood pressure at one-minute intervals.

The clinician should communicate appropriately with the participant regarding the purpose, time requirement and process of blood pressure measurement. Throughout the BP measurement, the clinician should keep the participant warm, relaxed and comfortable. The participant should be discouraged from reading, watching TV or talking, except to voice discomfort or confusion about instructions. The participant should be seated with both feet flat on the floor and with the back supported. Her right arm should be placed on the table in the proper position (i.e., at heart level with

the arm slightly flexed and the palms facing upward). The participant's arm should be bare to above the point of the shoulder.

MATERIALS NEEDED:

Dinamap blood pressure monitor
Blood pressure cuffs in four sizes (small adult, adult, large adult, thigh)
Gulik measuring tape

After the Dinamap monitor arrives at your site, please call the biomedical department in your clinic or hospital to assemble the monitor and perform the standard safety check. Prior to first use, the monitor will need to be charged overnight. If there is a problem with the Dinamap monitor, please call GE Medical Systems Technical Support at 1-877-274-8456 (follow the phone prompts leading to technical support).

1) ARM MEASUREMENT

The proper size cuff must be used to obtain accurate blood pressure (BP) readings.

- Ask participant to either remove her upper garment or to completely expose the right upper arm in order to perform the arm circumference measurement.
- In the standing position with the right forearm held horizontal, measure the arm length from the shoulder to the elbow. Mark the midpoint. (Arm circumference measurement is already being done in WIHS.)
- With the arm relaxed at the side of the body, place the tape measure horizontally, and draw snugly around the arm at the midpoint. Record the circumference.
- Consult the chart of arm circumference measurements and corresponding cuff sizes to choose the appropriate cuff. Do not rely on the markings on most BP cuffs – they may be incorrect!
- The left arm may be used if the BP is known to be higher in that arm, or in the presence of an anomaly or other circumstance prohibiting use of the right arm. Otherwise all BP measurements should be done on the right arm.

2) APPLYING THE BP CUFF

The cuff sizes used are:

Small adult: 17.1 – 25 cm
Regular adult: 25.1 – 33 cm
Large adult: 33.1 – 40 cm
Thigh: 40.1 – 50 cm

- Place the cuff directly on the skin, not over clothes.
- Palpate the brachial artery and place the midpoint of the length of the bladder over the brachial artery and the mid-height of the cuff at heart level.
- The lower edge of the cuff should be about one inch above the natural crease of the inner aspect of the elbow.
- Wrap the cuff snugly and secure firmly.
- The participant should be seated with both feet flat on the floor and with her back supported, and rest with her palm turned upward. Ask if the participant is relaxed, and, if necessary, help her to relax.

3. OBTAINING THE BP READINGS

The participant should be allowed to sit quietly for five minutes without talking. She should be seated comfortably, feet flat on the floor with her back supported. Ideally she should not have smoked or have had any caffeine within the 30 minutes prior to the BP determinations. After the five-minute waiting period, the clinician is to take three blood pressure measurements, with a one-minute wait between each measurement. The participant's arm should be passively raised overhead by the examiner for the first five seconds between each measurement. All measurements should come from the Dinamap.

1) Turn the Dinamap monitor on by pressing the blue "*on/off*" button on the lower right hand corner of the monitor. Then, press the light gray "*cycle*" button, which is below the green "*inflate/stop*" button on the upper right hand corner of the monitor. Make sure that the number "*1*" appears in the screen immediately to the left of the "*cycle*" button. This automatically sets the monitor to inflate, and then to re-inflate every one minute thereafter.
2) After the first blood pressure and pulse measurements appear on the screen, enter the systolic blood pressure, diastolic blood pressure and pulse onto the WIHS *Physical Exam* (*F07*) form. Please note that the pulse on the Dinamap monitor appears below the diastolic blood pressure.
3) After the cuff deflates, passively raise the participant's arm overhead for five seconds. Lower the arm gently.
4) Record the second blood pressure measurement and pulse, and again passively raise the participant's arm overhead for five seconds. Lower the arm gently.
5) Record the third blood pressure measurement and pulse, and then remove the cuff.

Always remember to turn off the monitor by pressing the blue button at the lower right hand corner of the monitor after all three blood pressure measurements are taken, otherwise the cuff will continue to inflate at one-minute intervals.

If, for some reason, you did not record the prior reading before the next blood pressure measurement was taken, you can press the gray "*history*" button after the blood pressure measurement is taken. The Dinamap monitor can store up to 25 blood pressure readings. If you would like to store just the three blood pressure measurements for the participant, clear all previous stored blood pressure measurements before taking the first blood pressure measurement. This can be done by pressing and holding the gray "*history*" button for two seconds. If you would like to print the blood pressure measurements, press the gray "*history*" button, followed by the gray "*print*" button, which is directly below the gray "*history*" button.

When the blood pressure is reported to the participant, the clinician should say, "Your average blood pressure today is …"

NOTE: If blood pressure is < 90/60 or > 140/90, refer participant to medical provider.

BP Classification	Systolic BP mmHg	Diastolic BP mmHg
Normal	< 120	and < 80
Prehypertension	120 – 139	or 80 – 89
Stage 1 Hypertension	140 – 159	or 90 – 99
Stage 2 Hypertension	≥ 160	or ≥ 100

Source: Seventh Report of the Joint national Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) Express; May 2003; *http://www.nhlbi.nih.gov/guidelines/hypertension/jncintro.htm*

III. URINE COLLECTION

Beginning with visit 31, urine samples will be collected for both pregnancy testing and storage in the WIHS central repository. Urine for pregnancy testing will continue to be collected at every visit; urine for repository storage will be collected annually, at even-numbered visits only. Additionally, urine for repository storage will be collected at the enrollment visit for 2011/12 and WIHS-V recruits.

2001/02 RECRUITS ONLY: An additional 20 ml of beginning stream urine is to be collected for the 2001/02 recruits at their baseline visit and is to be used for Chlamydia and Gonorrhea LCR testing. **The participant should not have voided for at least one hour prior to the collection of this specimen and collection of this specimen should occur prior to the pelvic exam.** This specimen is to be collected in a sterile container and must not be obtained as part of the clean voided specimen (CVS). Since this specimen is not a clean void, the participant does not need to follow the directions for a clean catch urine sample. Samples should be processed locally and in real time.

MATERIALS NEEDED:

- 1 or 2 sterile container(s) with screw cap
- 1 or 2 clean catch urine collection kit(s)
- Gloves for handling specimens

URINE FOR REPOSITORY STORAGE:

Beginning with visit 31, all participants should be asked to contribute a urine sample annually, at odd-numbered visits. Beginning with visit 36 and continuing throughout WIHS-V, urine collection for repository storage will be switched to occur at even-numbered visits. Urine collection can occur at any point during the study visit; for example, some sites may prefer to wait until near the end of the visit to limit the amount of time that a sample is at room temperature, while other sites may be able to ensure quick delivery to the lab if the sample is collected at the start of the visit.

Urine for repositing should be collected no more than two weeks after the core interview, and preferably on the same day as the core interview. A participant does not need to be asked to return to the WIHS clinic solely to provide a urine sample if a sample was not obtained on the same day as the core interview and she has no other reason to return to the clinic.

Provide the participant with a sterile collection cup and towelette. Instruct the participant how to collect a clean-catch, mid-stream urine sample:

1. First, please wash your hands.
2. Open the container and be careful not to touch the inside of the container or the lid.
3. Cleanse your genital area thoroughly with the towelette.
4. Urinate a small amount into the toilet, and then, without stopping, catch some urine into the container.
5. Close the container. Be careful not to touch the edges of the cup or the inside of the container.
6. Wash your hands.

Clearly label a Sarstedt 30ml polypropylene conical with a screw cap. The label should include at least the participant's WIHSID number, visit number, and time and date of collection. While standing at a sink and wearing appropriate PPE, transfer the urine to the conical. Do not send more than 30ml for processing; leftover urine can be discarded or used for pregnancy testing. The urine conical should be placed in a separate bag from blood collection tubes. If the lab will process samples the next day, ship on a cold pack separate from blood collection tubes.

PREGNANCY TESTING:

This sample of mid-stream urine is to be a clean voided specimen. The participant is to void into a sterile container. A small amount of urine can be taken from this specimen and a pregnancy test performed, if indicated. Pregnancy testing is required routinely at follow-up visits for every woman unless she is (1) s/p hysterectomy or (2) s/p bilateral oophorectomy or (3) greater than or equal to 50 years old. Pregnancy testing should be performed on women who self-report current pregnancy.

IV. GYNECOLOGICAL EXAM

A. EXTERNAL EXAM

NOTE: As of visit 23, the External Exam is optional and does not need to be recorded on the *F08*. Sites can decide if they wish to perform the External Exam on an individual or as needed basis.

Assist the participant into assuming the lithotomy position for pelvic exam. If the gynecological exam is not performed, the reason for the cancellation or postponement must be specified on *F08 (Gynecological Exam).*

The thighs, pubis, perineum and vulva will be visualized with the aid of an exam light. Lesions are to be described by number and type (e.g., vesicular, fissure-like or ulcerative).

NOTE: External lesions—located on the thighs, pubis, perineum or vulva—should be swabbed prior to the introduction of talcum powder or lubricant into the vagina.

B. LESION SWABS AND SLIDES

1. SPECIMEN COLLECTION FOR HSV CULTURE

   Baseline visit only (1994/95 and 2001/02 Recruits)—All ulcerations and fissures are cultured for HSV. After the removal of scabs or crusts, ulcers should be rubbed firmly with a sterile Dacron swab that is moistened with viral transport medium. In moist tissues, such as the vagina, the swab may be held in place for several seconds, and then rubbed against the lesion. Swabs are then placed in cold viral transport medium before drying occurs. The swabs are vigorously twirled in the medium, pressed against the side of the vial, and then discarded. The viral culture specimens are then sent to a local service laboratory.

   NOTE: Closed vesicles should be broken. A specimen should be obtained using a sterile swab.

2. SPECIMEN COLLECTION FOR SYPHILIS DFA SLIDE

   NOTE: Collection of slides for syphilis DFA was discontinued at visit 31.

   Baseline (1994/95 and 2001/02 Recruits) and follow-up visits through visit 30—DFA specimens are to be obtained for syphilis on all ulcerations and fissures.

C. VAGINAL SPECIMEN COLLECTION PROCEDURES

With gloved hands, palpate the external genitalia, and then insert a clean vaginal speculum. Gently insert an appropriately sized speculum, warmed with ONLY water, into the vagina until the cervix is visualized.

The appearance of the vaginal mucosa is described. The presence of ulcerations, warts, or other lesions is noted. The volume, color and character of vaginal fluid are assessed. Taking care to avoid contamination of the samples, obtain vaginal specimens on a swab from the vaginal wall, with cervical mucus.

1. VAGINAL SWABS

See **Section E** for the order of specimen collection during baseline and follow-up gynecological examinations.

Vaginal Swab #1:

The pH of the posterior vaginal pool is measured by applying a specimen, obtained from the posterior and lateral vaginal fornices with a Dacron swab, onto paper strips with a range of 4-7 (ColorpHast indicator sticks, EM Reagents, MCB Reagents, 480 Democrat Road, Gibbstown, NJ 08027). Call (609) 423-6300 for local distributor.

Vaginal Swab #2 on Culturette: (At Baseline only for 1994/95 and 2001/02 Recruits)

Specimens for the fungal culture are obtained using a swab from a sterile saline culturette. Rotate this swab on the left and right lateral vaginal walls. The swab is returned to the culturette, where the saline vial is broken, thus moistening the swab in its plastic tube. Send to local lab for storage and eventual Candida culture, as described in the Laboratory Manual Appendix I. Record a "V" on the Participant ID label to identify the source as vaginal.

Vaginal Swab #3:

NOTE: *Trichomonas culture is a site option.*

A sterile Dacron swab is used to sample the posterior vaginal pool for a trichomonas culture. After sampling, the swab is placed directly into Diamond medium that has been warmed to room temperature. Next, the swab shaft is broken off and the sample is gently agitated. Complete instructions for the incubation and reading of the culture are located in Laboratory Manual Appendix K.

Vaginal Swab #4 for Bacterial Vaginosis:

Using a sterile Dacron swab, obtain a sample for Gram Stain from the posterior vaginal pool. Roll the swab on a clean glass slide allowing it to air dry. On the frosted end of the glass slide, write with a lead pencil the WIHSID, the date the specimen was obtained and a "V" to identify the source of the slide as vaginal. Store the slide at room temperature.

Vaginal Swab #5 for Saline & KOH prep and Amine Test:

An additional sterile Dacron swab is used to obtain posterior vaginal fornix specimens for saline prep and KOH. The swab is pressed against two glass slides. Two drops of saline are applied to one slide, while two drops of 10% KOH are applied to the other slide. Immediately after mixing the specimen with KOH, the slide is placed close to the nose to detect whether there is a fishy amine odor (the presence of a fishy odor indicates a positive test). Cover slips are placed over the saline and KOH specimens.

At the end of the exam, perform the wet mount (Saline Mount) and the vaginal KOH prep (KOH Mount for Yeast). Record the results from the microscopic tests on *F08,* Question A44. Both preparations are to be examined under 100x and 400x power.

Saline prep:

Perform the microscopic examination of the slide looking for Clue cells (epithelial cells heavily studded with bacteria) and trichomonads. The presence of $\geq$ 20% of clue cells should be noted and indicates a positive test for clue cells. (Please see the WIHS Admin web site for the training instructions for reading vaginal wet mounts for clue cells: http://statepiaps.jhsph.edu/wihs/admin/clinical-training/Training_Evaluating%20Wet%20Mounts%20for%20the%20Presence%20of%20Clue.pdf).

The presence of motile trichomonads indicates a positive test for trichomonas. A wet preparation is considered negative for trichomonas if trichomonads are not seen when the entire coverslip area is viewed. Also assess whether there are increased WBCs (i.e., whether ratio of WBC:epithelial cells is greater than 1:1).

Vaginal KOH prep:

Perform microscopic examination of the KOH prep looking for fungal elements (hyphae or spores).

2. IMPORTANT NOTES

- At the end of the exam, the fungal culture specimen (culturette) should be transported to the local laboratory for processing and storage.
- Gram stain slides are to be placed in plastic slide holders, padded with tissue, and stored.

D. CERVICAL EXAM

Inspect the cervix visually. Assess for the presence of ectopy, warts, ulcers, polyps or friability. Assess for the presence or absence and character of any cervical exudate. Assess for evidence of a complete or partial hysterectomy. The absence or presence of cervical motion tenderness should be observed and recorded.

1. CERVICAL SWABS

See **Section E** for the order of specimen collection during the baseline and follow-up gynecological examinations.

Cervical Swab #1: Obtain swab at BASELINE only (1994/95, 2011/12, and WIHS-V Recruits)

A swab specimen of the cervical os is obtained for a *Neisseria gonorrheae* and *Chlamydia trachomatis* DNA probe. Remove the excess mucus from the cervical os and the surrounding mucosa using one of the swabs provided in the PACE specimen collection kit (Gen Probe Kit). Discard the swab, and place the second swab (1–1.5 cm) from the collection kit into the endocervical canal. Rotate the swab for 30 seconds. Withdraw the swab carefully to avoid contact with the vaginal mucosa. Insert the swab into the Gen-Probe transport tube (which contains a stabilizing fluid). Snap the shaft at the scored line, and cap the tube tightly. The tube may be stored at room temperature or in the refrigerator.

NOTE: This cervical swab is not obtained from women who do not have a cervix.

Cervical Swab #2 for LCR: Obtain swab only at Baseline, Visits 2 and 3 (1994/95 Recruits)

A second cotton swab specimen of the cervical os is obtained for the *Neisseria gonorrheae* and *Chlamydia trachomatis* ligase chain reaction. Use the wire shafted swab in the Abbott LCR collection/transport tube. Apply pressure to properly close the tube. Specimens should immediately be refrigerated and transported to the lab. Specimens are to be stored upright in -70C freezers in site local repositories for eventual testing.

NOTE: This cervical swab is not obtained from women with no cervix.

Cervical Swab #3 for HSV Swab and Syphilis DFA slide:

If cervical and/or vaginal ulcers or fissures are present, obtain a swab for HSV at the **Baseline (1994/95 and 2001/02 Recruits)** visit and a slide for syphilis DFA per instructions located above at **each study visit through visit 30**.

Cervical Swab #4 for HIV RNA Quantitation:

Beginning with visit 12 and at the **2001/02 recruits baseline visit**, a cervical swab for HIV RNA quantitation will be collected prior to collection of CVL. **Collection of this swab was discontinued beginning with visit 29.**

Cervical Swab #5 for HPV DNA:

Beginning with visit 31, a cervical swab for HPV DNA will be collected subsequent to collection of CVL and the Pap smear. A Dacron swab will be introduced into the cervical os, rotated 180 degrees five times within the endocervix, and then wiped along the external os. The tip of the swab will be placed in a 3.8 ml nunc tube containing viral transport media*, and its tip broken off at the notched break point. DNA precautions must be observed during this procedure, i.e., the portion of the Dacron swab that is left in the nunc tube must not touch surfaces (including hands) other than the cervix. Cervical specimens in viral transport media do not need to be immediately chilled, but should be frozen at -20°C within 72 hours, and transferred to storage at -80°C as soon as possible. If the participant does not have a cervix, the swab should be obtained from the vaginal pouch in the region of the cervix using the same methods.

* Qiagen Female Swab Specimen Collection Kit: 50 Dacron swabs and Specimen Transport Medium for cervical specimen collection (5123-1220) (http://www.qiagen.com/Products/Catalog/Assay-Technologies/Complete-Assay-Kits/HPV-Testing/digene-Accessories).

2. PAP SMEAR

Beginning with visit 38, some women will be able to have Pap tests done annually (once per year).

All women with less than or equal to five years (≤ 5 years) follow-up will have continue to have semi-annual Pap tests. This will include 2011-12 and WIHS-V recruits.

Women with greater than 5 years (> 5 years) follow-up can be switched to annual Pap tests if they are at low risk of pre-cancer, as assessed by:

- No history of CIN-2+ or HSIL at any time during WIHS follow-up. Women with a history of CIN-2+ /HSIL are considered at high risk for recurrence even years later.
- Prior two Pap tests were normal (at least one normal Pap during each of the prior two years).
    - Women who have an abnormal Pap test (ASC-US+) or abnormal histology (CIN-1+) return to semi-annual Pap tests until they have two normal Pap tests following or contemporaneous with normal colposcopy (if conducted). For example:
        - ASC-US Pap with CIN-1 colpo/histo → no treatment → normal Pap (#1) with normal coplo → normal Pap (#2) at next semi-annual visit → return to annual Pap
    - Women with missing data who don't meet the criterion of "at least one normal Pap test in each of the prior two years" return to semi-annual Paps until they do meet that criterion. For example:
        - Normal Pap → missed semi-annual visit → missed semi-annual visit → missed semi-annual visit → normal Pap test (#1) → requires Pap at next semi-annual visit and if normal (#2) can return to annual Pap

PROCEDURES:

If necessary, gently remove the vaginal debris with a large swab. The cytological smear specimen of the cervix is obtained by rolling a wooden spatula in two 360° rotations over the exocervix, and then rotating a cytological brush in the cervical os. Do not spread the spatula on the slide until the brush specimen is also ready. Spread the specimen onto the frosted side of a glass slide with the spatula and then, immediately following this procedure, spread the cytobrush specimen on top of the spatula specimen. IMMEDIATELY spray the slide with pump fixative.

CAUTION: Use of a cytobrush is contraindicated during pregnancy. If the participant is pregnant or up to eight weeks post-delivery or post-termination of a pregnancy, use a wooden spatula for two complete 360° rotations, and a cotton-tipped applicator for one complete 360° rotation. Place both on the same slide, and apply the fixative immediately. Allow to dry before inserting slide into a cardboard carrier.

NOTE: If cervix is not present, obtain a Pap smear from the vaginal cuff using a spatula.

NOTE: For women enrolled in the Cervical Cancer Screening Substudy (CCSS), see MOO Section 39 for Pap smear instructions.

SUPPLIES:

Beginning with WIHS visit 34, each WIHS site will obtain its own Pap smear collection kits and mailers.

- Pap kits with fixative included can be obtained from Fisher Scientific, catalog number: 14-372-36, Andwin Scientific No. 230110. A case of 500 kits is sold for $442.49; each kit contains one slide, cytology brush, plastic scraper, and fixative spray.
- **P7** (6 3/4" × 4 5/8" × 2 1/2") **Mailmaster® Mailing Boxes:** Reusable wire fasteners secure contents. Strong board protects valuable items and fragile components. Ideal for UPS, Parcel Post and storage. “PK” boxes are kraft. “P” boxes are white with 'wing-print' pattern. They have a minimum order of $100: 50/$0.83 each; 100/$0.74 each; 500/$0.66 each.

LABELING AND SHIPMENT:

a. Mark participant’s WIHSID number and month/year of birth on the frosted portion of the slide with a #2 pencil (the label will not last through processing).

b. Complete the UAB Medicine Cytopathology Requisition form (see **Appendix E** for example); one for each Pap smear shipped. Sites are encouraged to complete as much information in the electronic file as possible (e.g., site, clinician name and phone number) before printing to ensure that this information is legible.

   - Patient ID and Visit: Record the eight-digit WIHSID and the two-digit visit number, or affix a label containing that information.
   - Study Site: Record the study site number (i.e., Bronx = 1; Brooklyn = 2; Washington, D.C. = 3; Los Angeles = 4; San Francisco = 5; Chicago = 6).
   - Collection Date: Record the date that the Pap smear specimen was collected.
   - Specimen ID: Enter the ID from the local specimen database and associated label, if used.
   - Age: Record the participant’s age.
   - Date of Birth: Record the participant’s month, day, and year of birth in the format mm/dd/yyyy (e.g., 04/01/1999).

- Site Clinician name/#: Record the name and phone number of the clinician who collected the Pap smear slide.
- Physician Name/#: This field should always say "Strickler, H MD."
- Complete "Test and Source for Gynecologic Specimen Submitted." Indicate if the test will be a "conventional smear" or "other." And if the source is "cervical/endocervical," "vaginal," or "other." Specify if another location.
- Complete "Clinical Information and History." Enter the date of the participant's last menstrual period, and enter other information as to the participant's history. Indicate the number of weeks the participant is post pregnancy or post partum; whether she is post-menopausal, post-hysterectomy, or on hormone replacement therapy. Finally, indicate if the Pap smear is being done as a routine Pap or as a follow-up to an abnormal Pap smear.
- Enter any additionally relevant information in the "Relevant Clinical History" specify field.

c. Shipments to UAB should be made weekly, on Monday through Wednesday only (unless special arrangements are made with UAB to ship on a Thursday or Friday) using FedEx or another trackable courier. Tracking will be done by each of the six WIHS sites individually. With each shipment, one completed Cytopathology Requisition form must be sent for each individual slide.

   In addition, a manifest, listing all specimens to be sent, must be included in the shipment, as well as faxed to UAB prior to shipment: 205-975-7056. The manifest should include the following information:

   - Site number (1 – 6)
   - Shipper's contact person, phone number, email and mailing address
   - Total number of Pap smear slides in the shipment
   - A list of all eight-digit WIHSIDS, and associated visit numbers and dates of collection, for all slides included in the shipment
   - The local database tracking number of the external label, if used
   - The courier name, tracking number, ship date and expected arrival date

d. UAB will send the clinical report via fax within 10 days of receiving the Pap smear slide. Sites should send a query to UAB if a clinical report has not been received after two weeks. UAB will print C60 forms at their facility and will batch completed C60 forms to be shipped monthly to the sites for data entry.

NOTE: Beginning with visit 9, Dianon will forward Pap smear results on the WIHS Pap Smear study form *(C60)* directly to the WIHS sites for data entry.

NOTE: Beginning with visit 34, UAB will provide Pap smear reading instead of Dianon.

3. CERVICO-VAGINAL LAVAGE

Cervico-vaginal lavage is to be performed on all women whether or not the cervix is present. During the baseline visit of WIHS I through IV recruits, cervico-vaginal lavage is performed last. At WIHS V baseline as well as all follow-up visits, cervico-vaginal lavage is to be performed following the collection of vaginal swabs and before the collection of cervical swabs and Pap smear.

Using a syringe equipped with a 2-inch, 18 gage, angiocath type Teflon catheter, or a syringe equipped with one plastic transfer pipette (Fisher brand disposable graduated transfer pipette, Catalogue # 13-711-9A; pack of 500 @ $23), spray 10 ml of sterile normal saline against the cervical os and the exocervix. Using the syringe, aspirate the fluid from the posterior vaginal fornix, and transfer it to a 15 ml sterile polypropylene tube.

NOTE: If the pipette above is used to make the catheter tip for the syringe, cut the barrel of the transfer pipette just below the squeegee bulb to make the longest catheter possible.

If the volume recovered is less than 6 ml, a second lavage, using 5 ml of sterile normal saline, is to be conducted. Add the volume recovered to the 15 ml tube.

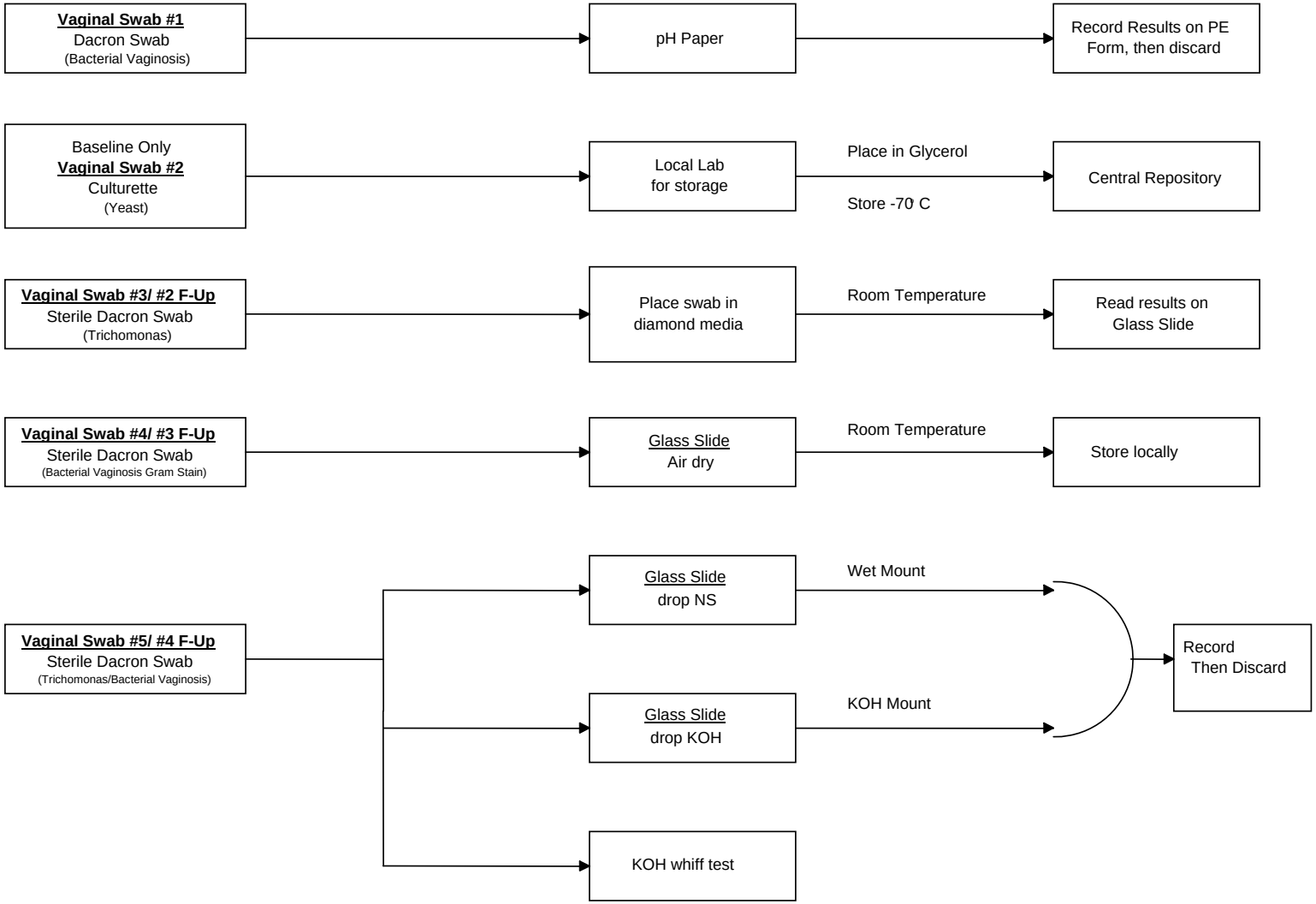
CVL is to be collected in one container and transported to the lab on ice or blue ice within one hour of collection, vortexed gently and aliquotted under a hood, under sterile conditions. Refrigerate the fluid at less than 10ºC if it will not be transported immediately to the lab; this will prevent microbial growth.

Flow Diagram

Vaginal Swabs (#1 - #5) Baseline
Vaginal Swabs (#1 - #4) Follow-Up (F-Up)

Supplies:

Culturette (1)	Sterile Dacron Swabs (3)
Dacron Swab (1)	pH Paper
KOH (10%)	Normal Saline
Microscope	Glass Slides (4)
Diamond Medium	



E. GYNECOLOGICAL EXAM ORDER OF SPECIMEN COLLECTION

1. BASELINE VISIT (1994/95 RECRUITS)

   a. Vaginal Specimens
      - Vaginal Swab #1 pH
      - Vaginal Swab #2 Fungal Culture
      - Vaginal Swab #3 Trichomonas Culture
      - Vaginal Swab #4 Bacterial Vaginosis Gram Stain
      - Vaginal Swab #5 T. vaginalis (wet mount), KOH prep, Amine Odor Test

   b. Cervical Specimens
      - Cervical Swab #1 GC & Chlamydia by Gen-Probe
      - Cervical Swab #2 Chlamydia by LCR (Abbott LCR Collection Kit)
      - Pap smear
      - Cervical Swab/Slide #3 HSV Culture/Syphilis DFA Slide
      - ***Cervical Vaginal Lavage***

2. BASELINE VISIT (2001/02 RECRUITS)

   a. Vaginal Specimens
      - Vaginal Swab #1 pH
      - Vaginal Swab #2 Fungal Culture
      - Vaginal Swab #3 Trichomonas Culture
      - Vaginal Swab #4 Bacterial Vaginosis Gram Stain
      - Vaginal Swab #5 T. vaginalis (wet mount), KOH prep, Amine Odor Test

   b. Cervical Specimens
      - Cervical Swab #4 HIV RNA Quantitation
      - ***Cervical Vaginal Lavage***
      - Pap smear
      - Cervical Swab/Slide #3 (prn) HSV Culture and/or Syphilis DFA Slide

3. BASELINE VISIT (2011/12 RECRUITS)

   a. Vaginal Specimens
      - Vaginal Swab #1 pH
      - Vaginal Swab #3 Trichomonas Culture (optional)
      - Vaginal Swab #4 Bacterial Vaginosis Gram Stain
      - Vaginal Swab #5 T. vaginalis (wet mount), KOH prep, Amine Odor Test

   b. Cervical Specimens
      - Cervical Swab #1 GC & Chlamydia
      - Pap smear
      - ***Cervical Vaginal Lavage***
      - Cervical Swab #5 HPV DNA

4. BASELINE VISIT (WIHS-V RECRUITS)

   a. Vaginal Specimens
      - Vaginal Swab #1 pH
      - Vaginal Swab #3 Trichomonas Culture (optional)
      - Vaginal Swab #4 Bacterial Vaginosis Gram Stain
      - Vaginal Swab #5 T. vaginalis (wet mount), KOH prep, Amine Odor Test

b. Cervical Specimens
- Cervical Swab #1 GC & Chlamydia
- ***Cervical Vaginal Lavage***
- Pap smear
- Cervical Swab #5 HPV DNA

5. FOLLOW-UP VISIT

a. Vaginal Specimens
- Vaginal Swab #1 pH
- Vaginal Swab #3 Trichomonas Culture (optional)
- Vaginal Swab #4 Bacterial Vaginosis Gram Stain
- Vaginal Swab #5 T. vaginalis (wet mount), KOH prep, Amine Odor Test

b. Cervical Specimens

VISITS 2 AND 3
- ***Cervical Vaginal Lavage***
- Cervical Swab #1 Chlamydia by LCR (Abbott LCR Collection Kit)
- Pap smear
- Cervical Swab #2 Syphilis DFA Slide

VISITS 4 THROUGH 11
- ***Cervical Vaginal Lavage***
- Pap smear
- Cervical Swab Syphilis DFA Slide

VISITS 12 THROUGH 28
- Cervical Swab #4 HIV RNA Quantitation
- ***Cervical Vaginal Lavage***
- Pap smear
- Cervical Swab Syphilis DFA Slide

VISITS 29 AND 30
- ***Cervical Vaginal Lavage***
- Pap smear
- Cervical Swab Syphilis DFA Slide

VISITS 31 AND UP
- ***Cervical Vaginal Lavage***
- Pap smear
- Cervical Swab #5 HPV DNA

F. UTERINE EXAM

NOTE: As of visit 23, the Uterine Exam should be performed once per year, only at even visits.

Next, the uterine exam is performed. The absence or presence of uterine tenderness or enlargement and is observed and recorded.

G. ADNEXAL EXAM

NOTE: As of visit 23, theAdnexal Exam should be performed once per year, only at even visits.

Next, the adnexal exam is performed. The absence or presence of adnexal tenderness or enlargement and is observed and recorded.

H. RECTAL EXAM

NOTE: As of visit 23, the Rectal Exam is optional and does not need to be recorded on the *F08*. Sites can decide if they wish to perform the Rectal Exam on an individual or as needed basis.

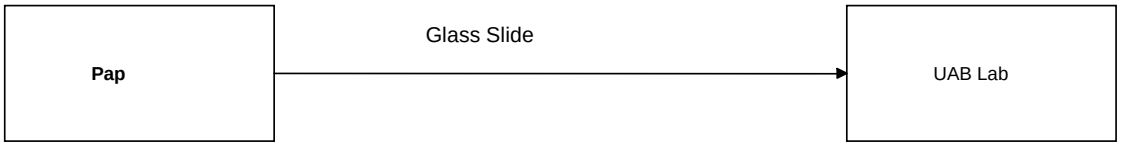
The external anus will be examined for the presence of visible warts, hemorrhoids, discharge and ulcerations. Lesions, such as ulcers or warts, are recorded on the lesion form, and diagnostic testing is performed as described in the preceding sections.

NOTE: If the participant is HHV-8 positive and enrolled in the HHV-8 Substudy, the clinician will collect an anal swab during the Gynecological Exam at visit 10. See **MOO, Section 16** for the complete HHV-8 Substudy Protocol.

Flow Diagram
Pap Smear

Supplies:

Pap Smear Kit

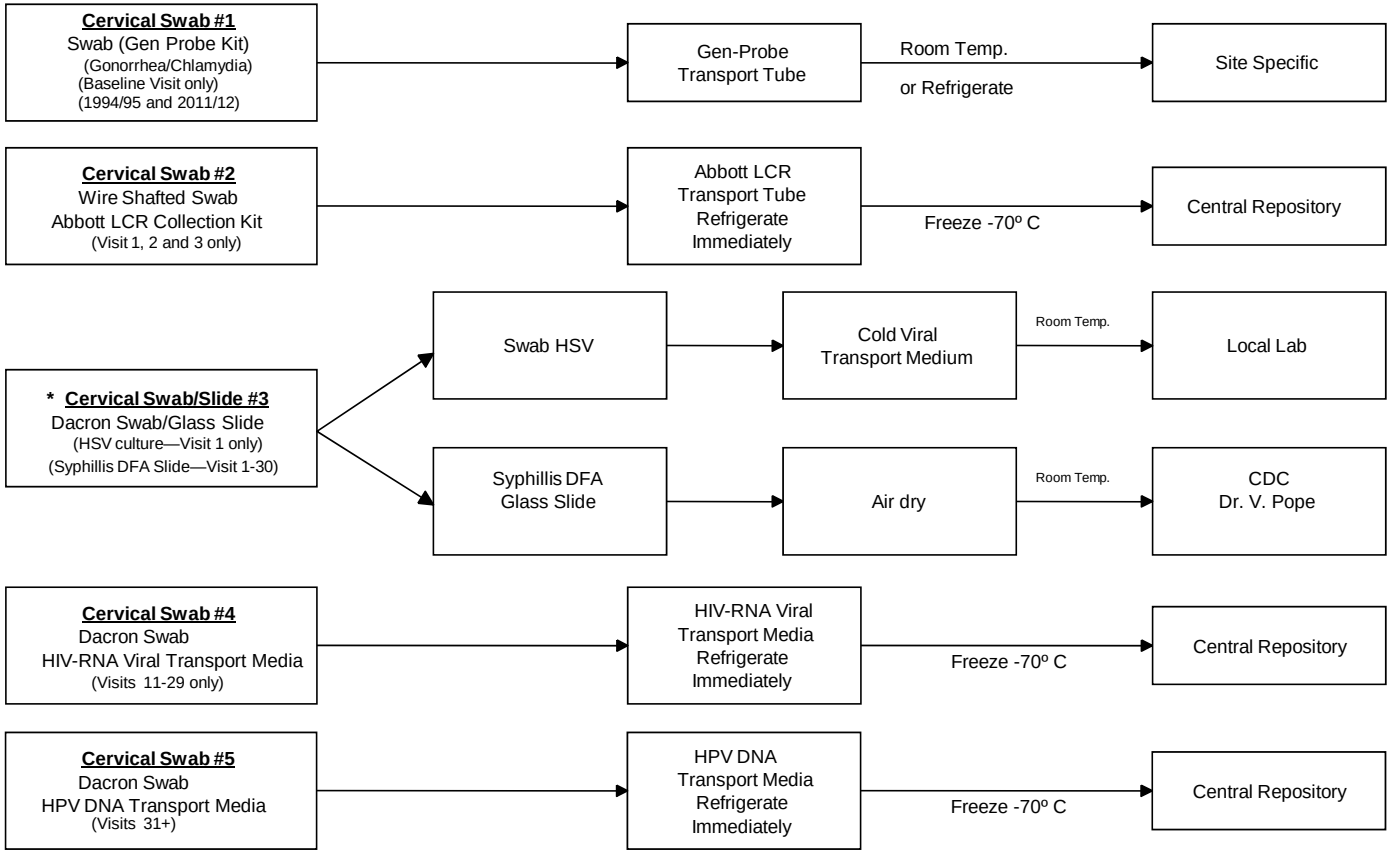


NOTE: If NO cervix is present, obtain pap smear from vaginal cuff.

Flow Diagram

Cervical Swab (#1 – Visit 1 only)
Cervical Swab (#2 – Visits 1, 2 and 3 only)
Genital Ulcers (#3 – Visits 1-30)
Viral Load (#4 – Visits 12- 28)
HPV DNA (#5 – Visits 31+)

Supplies:
Gen Probe Kit Swab (1)
Dacron Swab (2)
PACE Specimen Collection Kit (1)
Abbott LCR Kit (1)
Glass Slides
Cold Viral Transport Media
HIV-RNA Viral Transport Media
HPV DNA Transport Media

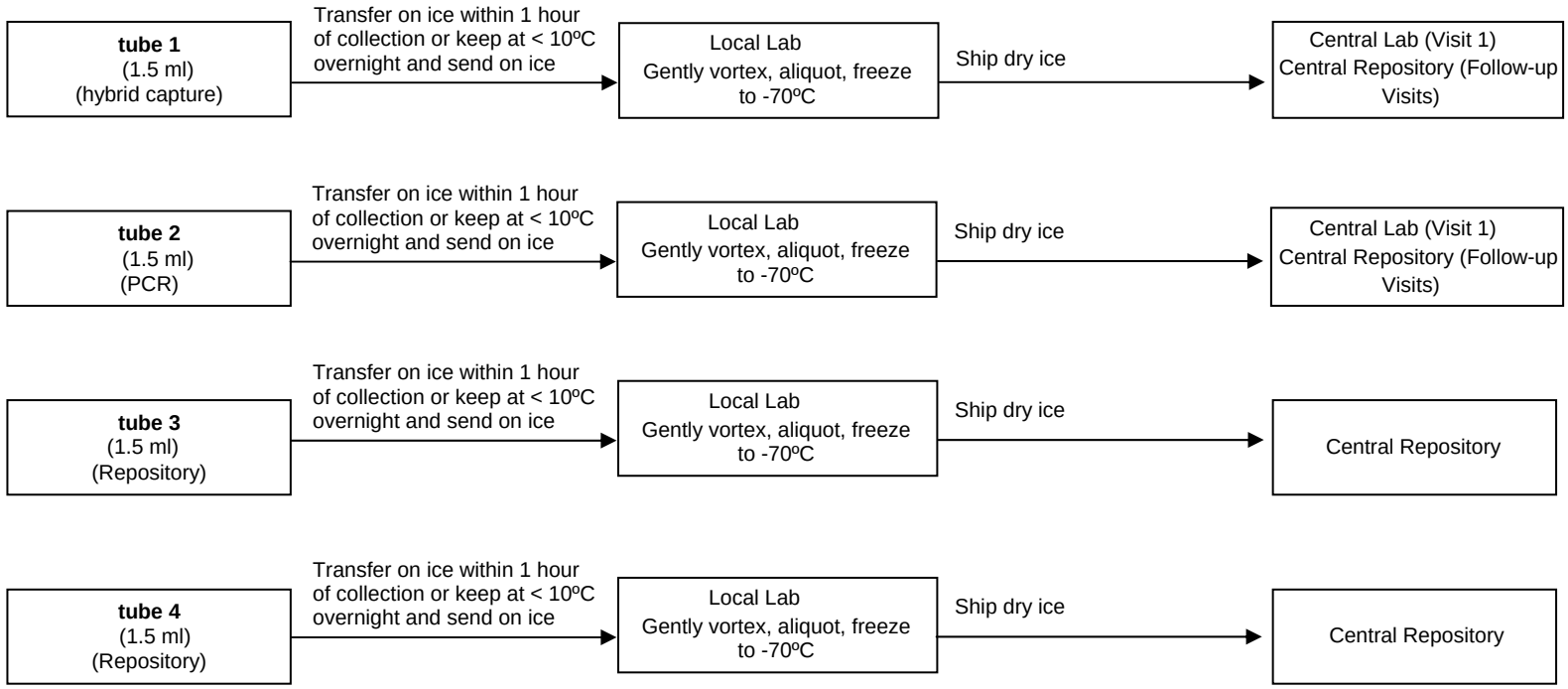


* All ulcerations and fissures will be cultured for HSV of vaginal and/or cervical lesions.
* DFA specimens for syphillis will be obtained from vaginal and/or cervical lesions.

Flow Diagram
Cervico-Vaginal Lavage Samples

Supplies:

Normal Saline (10 ml)
Syringe
2-inch 18 gage angiocath type teflon catheter
Pipett
Sterile polyproylene tube (15 ml)



NOTE: At Baseline and first two follow-up visits, aliquot 7 ml of CVL into 7x 1 ml aliquots.

V. COLPOSCOPY/BIOPSY/TREATMENT

This section describes the follow-up procedures to be triggered by any abnormal Pap smear as based on the first read performed by the pathologist. The colposcopy should be done on the same day as the physical exam. However, if this is not possible or not indicated until receiving the Pap smear results at a later date, the colposcopy should be performed within one month, and no longer than two months (60 days), from the WIHS core visit date.

When indicated and clinically feasible, a colposcopy will be performed on all the women in the study regardless of whether or not a colposcopy was performed prior to the WIHS visit.

It is preferable to have all WIHS colposcopies performed by a WIHS-trained colposcopist. However, as this is not always possible, a question has been added to form *L14 (Colposcopy Results Form)* to indicate whether the colposcopy was performed by a WIHS colposcopist or an "outside" colposcopist. If performed by an outside colposcopist, the site should obtain consent for medical record abstraction, obtain the colposcopy results and report these results on the *L14, L15 (Laboratory – Pelvic Exam Studies, Histopathology Report), L16 (Laboratory – Pelvic Exam Studies, Treatment Form),* and *COLPO (Colposcopy Tracking)*, as appropriate. The examiner will be responsible for referring women appropriately for colposcopy or treatment when clinically indicated.

NOTE: Some sites may select additional criteria as an indication for performing a colposcopy.

A. COLPOSCOPY / BIOPSY PROTOCOL

1. INDICATIONS FOR COLPOSCOPY

Group 1

If Pap is normal and no lesion suspicious for condyloma/dysplasia/cancer is visible by gross inspection, then repeat Pap q6mo.

Group 2

1. If Pap is normal but visible lesion suspicious for condyloma is visible or if participant has a Pap ASCUS after no prior colposcopy, then refer for colposcopy of the cervix and vagina. If participant has either (1) stable visible condyloma after prior colposcopy/biopsy or (2) repeat and sequential Pap ASCUS after prior negative colposcopy, then colposcopy may be performed annually, rather than semiannually after every ASCUS result, at colposcopist's discretion.
2. If no significant lesion is found at colposcopy, then return to Group 1 (i.e., Pap but not colposcopy q6mo).
3. If lesion found and treatment other than hysterectomy performed, then repeat colposcopy after six months. If normal, return to Group 1.
4. If lesion found and hysterectomy performed, then return to Group 1.
5. If lesion found but no treatment is indicated or if treatment is indicated but not performed (e.g., patient declines), follow with colposcopy q6mo.

   a. If lesion regresses completely, then return to Group 1.

   b. If lesion persists unchanged, then follow with colposcopy but not biopsy q6mo.

   c. If lesion changes, repeat biopsy.

Group 3

1. If Pap LSIL, then refer for colposcopy of cervix and vagina.
2. If no significant lesion found at colposcopy and colposcopy satisfactory, return to Group 1.
3. If no significant lesion found at colposcopy but colposcopy unsatisfactory, repeat q6mo x 2.

a. If no HSIL/Ca on Pap and colposcopy remains normal, return to Group 1.

b. If no HSIL/Ca on Pap but lesion seen, biopsy and manage as Group 2.

c. If HSIL/Ca on Pap, manage as Group 4.

Group 4

1. If Pap HSIL, AGUS or cancer, then refer for colposcopy of cervix and vagina.
2. If no significant lesion found at colposcopy or if colposcopy unsatisfactory, perform conization if participant consents. Repeat colposcopy/Pap after six months.
   a. If negative and Pap negative, return to Group 1.
   b. If positive, biopsy and manage as per above protocol.
   c. If participant refuses conization, then repeat colposcopy after six months.
3. If lesion present and colposcopy satisfactory, perform loop excision or cone. Repeat colposcopy/Pap after six months.
   a. If negative and Pap negative, return to Group 1.
   b. If positive, biopsy and manage as per above protocol.
   c. If patient refuses conization, then repeat colposcopy after six months.
4. If no treatment given, follow with colposcopy q6mo with biopsy only if Pap worsens or lesion characteristics change.

Notes:

- If any biopsy shows cancer, refer participant to gynecologic oncologist.
- ECC not required with colposcopy, but may be performed at colposcopist's discretion if colposcopy satisfactory and any Pap result, or if Pap ASCUS and colposcopy unsatisfactory. ECC required if Pap AGUS/LSIL/HSIL/cancer and colposcopy unsatisfactory, except during pregnancy.
- For Group 2, significant lesions include koilocytosis, HPV effect, HPV change, condyloma, dysplasia, CIN/VaIN, cancer. Insignificant lesions include metaplasia of any type, inflammation, cervicitis, and atypia not associated with koilocytosis or HPV.
- For Group 3, significant lesions are dysplasia, CIN or VaIN 2-3, and cancer. Insignificant lesions are those that do not require treatment, including those listed for Group 2 plus koilocytosis, HPV effect, HPV change, condyloma, and CIN or VaIN 1.
- Conization can be performed with cold knife, laser, or wire loop.
- Endometrial biopsy should be considered for women with AGUS at risk for endometrial cancer (abnormal bleeding, obesity, and/or age $\geq$ 35 years).

2. SCHEDULING

a. A colposcopy will be performed at the time of the research exam whenever possible.

b. For sites that need to schedule participants for colposcopy, a colposcopy will be performed within one month (no longer than two months) from the WIHS core visit.

c. WIHS participants do not need to come back in for a repeat Pap smear for Pap smears that have a result of "satisfactory" or a "satisfactory, but limited..." for specimen adequacy, and NO endocervical cells present. A Pap smear is to be repeated at the participants' next scheduled visit unless there is another indication.

d. If a woman has had a complete colposcopy and biopsy within three months of the WIHS core visit and the Pap smear and biopsy results are available, another biopsy only needs to be performed if the biopsy results and the current findings on colposcopy differ. If a biopsy is NOT performed during the WIHS core visit, record the pathology results for the biopsy obtained within the past three months on the WIHS *Histopathology Report Form (L15)*.

3. PROCEDURES

   a. Colposcopy must be performed on the cervix and the vagina.

   b. The vulva may be examined by inspection only, unless a visible vulvar or perianal lesion is present.

   c. Anal colposcopy using an anoscope may be performed as clinically indicated and feasible.

   d. If no lesions are seen upon performing colposcopy, Lugol's staining of the vagina may be performed.

B. PROTOCOL FOR ADMINISTERING THE *TREATMENT DYSPLASIA FORM (L16)*

1. Any participant with an abnormal Pap (ASCUS or higher, per the Colposcopy Protocol) will be referred for a colposcopy. If the participant is getting a "routine" WIHS colposcopy, skip to #3.

2. If the colposcopist to whom the participant is referred is not a WIHS investigator, then the study staff should obtain the name, address, etc., of the colposcopist at the time that the abnormal Pap was reported. This information can be recorded on the *ATC* form to aid the abstractionist in obtaining the participant's medical records.

3. After the scheduled colposcopy, the study staff should contact the colposcopist to ascertain whether treatment has been rendered, based on colposcopic impression. If so, it is at this point that the *Treatment Dysplasia Form (L16)* should be filled out. The colposcopist should be recontacted at a time when the histology result is available, in order to:

   a. allow completion of treatment form and

   b. find out if further treatment is anticipated.

   If further treatment is recommended, the colposcopist must be contacted after each scheduled therapy.

4. If treatment is recommended based on either:

   a. a routine WIHS colposcopy that revealed an abnormality or

   b. a colposcopy performed because of an abnormal Pap (see #1),

   and treatment is to be performed by an individual other than that colposcopist, study personnel should obtain the name, address, etc., of the physician to whom the participant is referred. That physician should be contacted to ascertain information required in order to complete the *Treatment Dysplasia Form (L16)*.

5. Whenever the form is completed, the source(s) of the data should be noted (i.e., contact with clinician, chart review, participant history, etc.).

6. The form should be completed for *every* treatment date, even if the treatment dates are for the same lesion. If a participant has multiple lesions, one form can be used for all the treatments that take place on the same day (e.g., a cervical and a vulvar lesion). A rare exception to this would be a participant with multiple lesions at a single site (e.g., cervix) that require separate treatments. In the latter circumstance, additional forms will need to be completed to document treatment.

NOTE: For repeated treatments, complete one *Treatment Dysplasia Form (L16)* per treatment cycle. For example: If there are five treatments for Condyloma at one site, complete one *L16*. If there is another site involved, complete a second *L16*.

VI. TUBERCULIN AND ANERGY SKIN TESTING

All women require PPD/anergy skin testing at enrollment, regardless of history, except in cases where:

1. there is a history of severe skin reaction or sloughing, or
2. the woman is currently being treated with TB medications.

The PPD/Anergy skin testing is to be administered at Visit 1. However, for the WIHS Protocol, PPD/Anergy skin testing can be done at the six-month follow-up visit (Visit #2) if:

1. skin testing is not able to be administered at Visit 1;
2. the participant did not come back for the reading of her skin tests; or
3. the participant came back late for the reading of her skin tests and the TB test result was unreliable and the TB test was not replanted.

PPD testing will be performed on all participants at enrollment. Negative PPD reactors **only** will be tested annually at odd-numbered visits. Anergy skin tests will be performed on **all** participants at both enrollment AND annually at odd-numbered visits through visit 11. The PPD and Anergy skin testing should be placed together on the same visits. Women who have a positive PPD at the WIHS Baseline visit are still required to have Anergy skin testing annually at odd-numbered visits through visit 11.

PPD/anergy skin tests will be read between 48 and 72 hours. If women do not return for the reading of the results, the data will be considered missing. For compliance with the WIHS protocol, the PPD/anergy skin tests do not need to be replanted. In these cases, PPD/anergy skin testing is to be placed at the next odd visit (Visit #2).

NOTE: For 2001/02 recruits, PPD testing will be performed as described above. Anergy testing will not be performed for the 2001/02 recruits. Neither PPD nor anergy testing will be performed for 2011/12 or WIHS-V recruits.

A. IMPORTANT NOTES

- There is no exclusion window from prior testing, i.e., even women who were tested in the one or two weeks prior to WIHS enrollment must be retested.
- Both the PPD and Anergy skin testing will be on the same schedule.
- The PPD/Anergy Panel may be placed two to three days prior to a study visit, so that skin testing can be read on the same day as the study visit.
- Mumps antigen should not be administered to anyone with a history of allergy to eggs or egg products.
- Anergy panel placement was discontinued after visit 11.
- PPD testing was discontinued starting with visit 17.

B. TUBERCULIN AND ANERGY SKIN TESTING PROCEDURES

1. The skin tests should be applied on Monday, Tuesday, Wednesday or Friday because they need to be read two to three days later. The skin tests should NOT be placed on Thursday, unless special arrangements have been made to read on a weekend.
2. If possible, the participants should be given a return appointment two days later to have their skin tests read. This allows for recalling participants who miss the visit to come in on the third day.
3. Place the tuberculin test (5 TU PPD) in the same place on every participant (e.g., the left upper forearm), so that you will know where to read it later. Do not place the test over a vein or in a place where there is a scar or rash.
4. Place each anergy test (mumps, tetanus and Candida) in the same place on every participant. Record where you placed each test to assist reading the results. For example:

PPD	upper left forearm
Mumps	lower left forearm
Candida	upper right forearm
Tetanus	lower right forearm

   Do not place the tests over a vein or in a place where there is a scar or a rash.
5. For each skin test, use a 2001/02 tuberculin (1.0 cc) syringe with a 26 or 27 gauge needle. Using sterile technique, draw up 0.1 cc of skin test solution (PPD, mumps, Candida, and tetanus**)** in the syringe. You may draw up a bit more and squirt out the excess, but it is important to test the participant with <u>exactly</u> 0.1 cc of solution.
6. Insert the needle with the bevel up. Lift the needle up while it is in place to check its position. If you can lift the tip of the needle up and draw up the skin with it, the needle has not gone too deep. If you still see the bevel of the needle at the entry site, the needle has not gone deep enough and the solution will leak out.
7. Inject the solution intradermally (into the skin), not subcutaneously (under the skin). When you inject the solution, you should see a definite circular thickening of the skin (a "wheal") where the fluid has gone. If you cannot see and feel the wheal, the fluid has gone too deeply and you must repeat the injection.
8. Skin testing is designed to test delayed-type hypersensitivity (DTH) reactions. Some persons may have immediate-type hypersensitivity reactions to test antigens, with local redness and/or swelling within minutes or hours of testing. These reactions, which occur less than 48 hours after application of the tests, do not provide useful information and should be ignored. Do not interfere with DTH reactions.
9. When reading the test, feel with your finger for <u>induration</u> (thickening, localized swelling, or hardening) of the skin. If you see redness, ignore it. If you cannot feel any thickening or hardening, the test is negative (0 mm). If you feel a distinct ridge at the edge of the thickened area, mark the ridge with a ballpoint pen at the edges on all four sides of the thickened area.
10. Measure the marked transverse induration (parallel to wrist / elbow creases). Record the diameter in the nearest <u>whole millimeter</u>. Add leading zeros where necessary (i.e., record "007" rather than "7"). If there is no induration, record "000."
11. If the induration at the site of the TB skin test (PPD) measures 5 mm or more for seropositive women (10 mm or more for seronegative women), notify the clinician for further immediate evaluation. Depending on their medical history and current symptoms, persons with positive skin tests may be considered to have the tuberculosis infection, and may need to be urgently or routinely referred to the health department or a medical facility for diagnosis and/or treatment.

NOTE: Seronegative women that have a PPD reading between 5 mm and 10 mm of induration require a referral for clinical evaluation and or treatment.

12. The test should be read for participants who return two or three days after the test is applied. Those who return in less than two days should not have their test read, and should be instructed to return at an appropriate time for reading.

    If participants return within four to seven days after their tests were applied, read the test and record the results. If the TB test is positive ($\geq$ 5 mm for HIV seropositives, $\geq$ 10 mm for HIV seronegatives), the result is considered reliable, even though the test was read late. **If the TB test is not positive, the result is not considered reliable.** These participants, and those who return more than seven days after their tests were applied, should be offered retesting, and told that they need to return in another two or three days to have their second test read. The importance of coming at the proper time should be re-emphasized. However, if a replant does not yield a reliable result, the WIHS Protocol allows for the PPD/Anergy skin tests to be placed and read at the six-month follow-up visit.

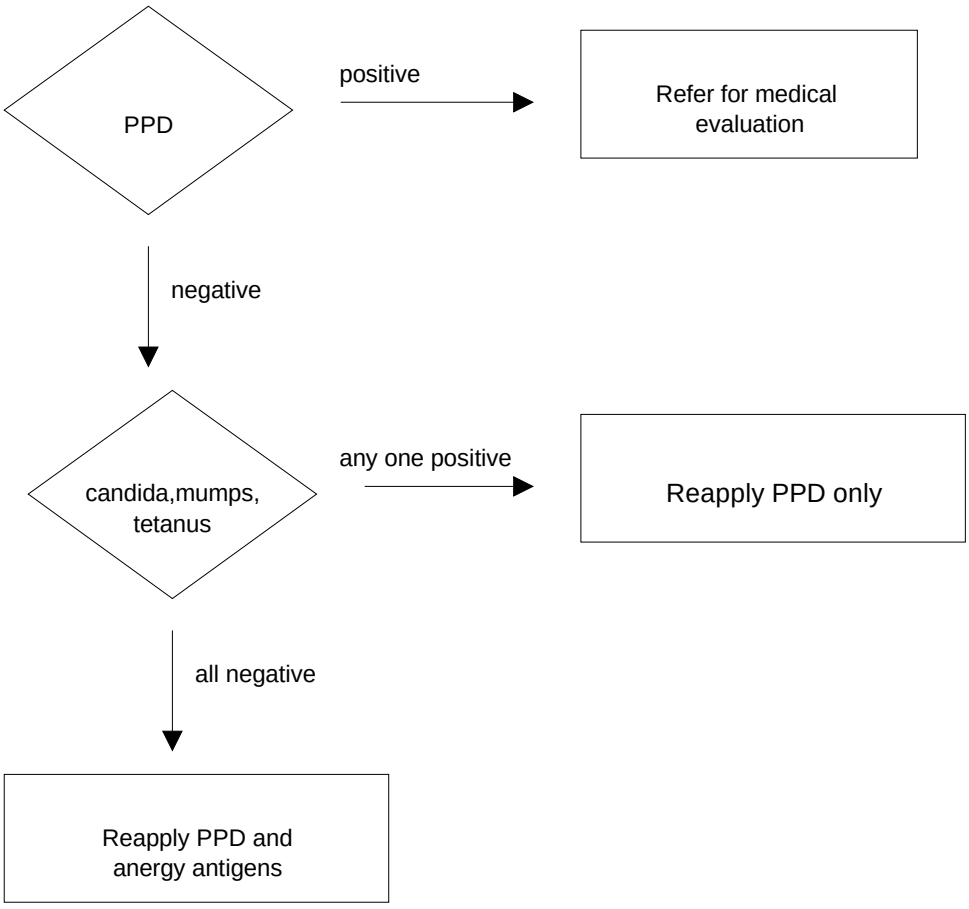
Time	Read test?	Other
Less than 2 days	NO	Instruct participant to return on 2nd or 3rd day after test
2–3 days	YES	If PPD positive, refer for medical evaluation
4–7 days	YES	If PPD positive, refer for medical evaluation If PPD NOT positive, reapply tests and instruct participant to return in 2–3 days
8 or more days	NO	Reapply tests and instruct participant to return in 2–3 days

13. For determining the possible need to replace the antigen tests, reactions to anergy antigens (mumps, tetanus, candida) are considered positive if the transverse diameter is 2 mm or greater.

14. If you do apply a second tuberculin test, use the following table to decide whether to reapply the anergy tests also.

Time	PPD reaction	Anergy reaction	Reapply anergy tests?
4–7 days	Positive	(does not matter)	NO
	Negative	Any one positive ($\geq$ 2 mm)	NO
		All negative	YES
8 or more days	(does not matter)	(does not matter)	YES

For participants who come back 4 - 7 days after skin test application:



15. Do not apply a second skin test in the same place as a previous test. Place the second test at least 5 cm (2 inches) from the previous test. Carefully record exactly where you placed the test (you may use the clinical mapping addendum) so you will be able to read it later.
16. If a participant fails to return to have her second test read, you may apply the test a third time, particularly if the participant was in jail or has another good explanation for failing to show up. We would like to maximize our chances of reading tests on everyone, but you should not apply the test a third time if you feel the participant is unlikely to return for reading at the proper time.

C. PREPARATION OF TETANUS SKIN TEST ANTIGEN

1. Be sure to use careful, sterile technique with each transfer.
2. You should have a vial of Tetanus Toxoid and a 5 ml vial of Albumin Saline. Check the expiration dates on each vial. The Human Serum Albumin Diluent (Albumin Saline) to be used is 0.03%.
3. Transfer 1.0 ml of Tetanus Toxoid to the 5 ml vial of Albumin Saline. Shake the Tetanus/Albumin vial to mix the contents thoroughly.
4. Be sure to label the Tetanus/Albumin vial by recording the date, and indicating that the vial now contains tetanus toxoid.
5. In general, no vial of any of the skin test antigens should be kept longer than 30 days after you have begun using it to perform skin tests. Store each as directed in the package inserts. Undiluted

Tetanus Toxoid and Albumin Saline may be kept for up to three years (check package insert and vials for expiration dates).

D. ANERGY SKIN TEST MATERIALS

MUMPS

Manufacturer:	Connaught
Phone number:	(800) 822-2463 x4467 Ann x4360 Kidren
Product name:	MSTA (Mumps Skin Test Antigen)
How Supplied:	1 ml vial (10 tests)
Product cost:	$28.55

CANDIDA

Manufacturer:	Allermed Laboratories, Inc.
Phone number:	(619) 292-1060
Product name:	Candin
How Supplied:	1 ml vial (10 tests)
Product cost:	Provided by Allermed Laboratories, Inc. Attn. Dr. H.S. Neilson, Jr.

TETANUS TOXOID

Manufacturer:	Connaught
Phone number:	(800) 822-2463 x4467 Ann x4360 Kidren
Product name:	Tetanus Toxoid USP (fluid, not aluminum-absorbed)
How Supplied:	7.5 ml vials (375 doses, diluted 1:5 in albumin)
Product cost:	$9.80

HUMAN SERUM ALBUMIN

Manufacturer:	Miles (Hollister/Stier)
Phone number:	(800) 992-1120 1=order 3=technical
Product name:	Human Serum Albumin Diluent (Albumin Saline) 0.03%
How Supplied:	Package of 25, purchase 5 ml vials with 4 ml albumin
Product cost:	$27.98 (25-5 ml vials)

E. ANERGY TESTING IN HIV-INFECTED PERSONS AT INCREASED RISK FOR LATENT OR ACTIVE TUBERCULOSIS

What is anergy?

Anergy is the inability to mount a delayed-type hypersensitivity (DTH) response to a battery of common skin test antigens. Anergy represents the suppression of cellular immunity.

Why should we be concerned about anergy?

Recent reports have suggested that the sensitivity of the tuberculin (PPD) skin test may be substantially reduced in asymptomatic persons with the Human Immunodeficiency Virus (HIV) infection. More than 10% of TB/HIV dually-infected persons may have a negative skin test when tested with tuberculin. These "false negative" responses make decisions concerning tuberculosis preventive therapy problematic. Because of this, the Centers for Disease Control is now recommending that persons infected with HIV, and at increased risk of infection with M. tuberculosis, be evaluated for DTH anergy in conjunction with PPD testing.

What can cause anergy?
While we are primarily concerned with anergy in persons infected with HIV, other diseases or conditions can also cause suppression of DTH responses. These include:

- viral infections (measles, mumps, polio)
- bacterial infections (typhoid fever, pertussis, brucellosis, leprosy, overwhelming tuberculosis)
- live virus vaccinations (measles, mumps, polio)
- chronic renal failure
- malnutrition
- drugs (corticosteroids and other immunosuppressive agents)
- diseases affecting lymphoid organs (Hodgkin's disease, lymphoma, chronic lymphocytic leukemia, sarcoidosis)
- age (newborn or elderly participants)
- stress (surgery, burns, mental illness)

How can we test for anergy?
Anergy is usually assessed by testing with a panel of skin-test antigens to which most healthy people would be sensitized and expected to react. These include bacterial, viral, and fungal antigens, such as tuberculin, histoplasmin, mumps, tetanus toxoid, Candida, coccidioidin, and trichophyton. The most commonly used antigens are tuberculin, mumps, tetanus toxoid, and Candida. The CDC recommends testing with at least two DTH skin-test antigens, in addition to tuberculin. Tests administered by the standard Mantoux technique are recommended.

What is the Mantoux technique?
The Mantoux skin test is performed by the intracutaneous injection of 0.1 ml of an antigen into either the volar or dorsal surface of the forearm. The use of skin area free of lesions and away from veins is recommended. Alternate sites, such as the upper back or shoulders, may be used when the arms are not suitable. The injection is made with a short (1/4 to 1/2 inch), bluntly beveled, platinum (26-gauge) or steel (27-gauge) needle with a glass or plastic tuberculin syringe. The injection should be produced when the prescribed amount of fluid (0.1 ml) is injected intracutaneously. Multiple tests given on the same arm should be placed at least 5 to 6 cm (2 to 2 1/2 inches) apart.

When do you read skin tests?
Tests should be read the second or third day after injection (48 to 72 hours), the time when the induration is usually most evident. Definite palpable and measurable induration of >5 mm may be read up to one week following testing.

How do you read skin tests?
The reading should be conducted in a good light, with the forearm slightly flexed at the elbow. The basis of reading is the presence or absence of induration, which may be determined by inspection (from a side view against the light, as well as by direct light) and by palpation. The diameter of induration should be measured transversely to the long axis of the forearm, and be recorded in millimeters, not just as “positive” or “negative” (i.e., no reaction would be 00 mm). Disregard erythema, since this is not an indication of delayed-type hypersensitivity (DTH).

What is induration?
The DTH induration is an immune response to a particular antigen, involving lymphocyte sensitization and cellular infiltration. It is a firm, raised, usually round bump at the site of the injection.

What is erythema?
Erythema is an acute inflammatory reaction caused by vasodilation and congestion of the capillaries (redness).

How do you define a positive test?
Most manufacturers of skin test antigens suggest induration of 5 mm or greater as the definition of a "positive" test. While this degree of induration represents the "normal" DTH function, responses between 2 and 5 mm should be considered evidence of some DTH competency. For example, an individual with a 3 mm induration to tetanus toxoid would have diminished DTH function, but would not be considered anergic.

What other factors could influence a skin test response?

- Tester variation – Persons performing the skin tests should be trained in the Mantoux technique. If the needle is inserted too deeply, the reaction will be difficult to palpate or see. If it is inserted too shallow, the antigen will leak out, and the amount given is insufficient. If either occurs, the test must be repeated at another site. In addition, antigens injected too close to one another may result in overlapping areas of induration, thus making an accurate measurement of reaction sizes difficult.
- Reader variation – The reader must be able to distinguish between induration and erythema, read the test at the appropriate time (48 to 72 hours), and record the induration in millimeters. Recording "positive" or "negative" is not acceptable!
- Poor storage and handling of the products – All antigens should be stored according to the manufacturers' instructions. This is usually in a cool dark place, such as a refrigerator. Antigens should be drawn into the syringe just prior to their use to avoid contamination or absorption of the antigen onto the plastic syringe.

How do I handle testing with multiple antigens?
It is important to establish a consistent scheme for administering the antigens. For example, always give the same antigen in the same location (i.e., PPD on the left arm, the other on the right). To avoid mixing up the antigens, give the test immediately after drawing the antigen into the syringe. If you need to fill all of the syringes at one time, be sure that the syringes are properly labeled.

What antigens are recommended for anergy testing?

1. TETANUS TOXOID

   DTH reactivity to tetanus toxoid is dependent on prior immunization with the toxoid. This antigen is particularly useful, as it is given as part of the standard immunization schedule in the United States. The antigen may have limited use for testing individuals born in countries where such vaccination practices are not followed.

   Antigen: Fluid toxoid should be used for DTH testing; Aluminum phosphate absorbed toxoid is not recommended. Tetanus toxoid is not specifically licensed for DTH testing; and, therefore, information concerning such testing is not available from the manufacturer. However, the antigen is known to elicit DTH responses, and can be used for anergy testing.

   Concentration: 1:5 dilution in human serum albumin diluent (one part toxoid to four parts diluent). Once diluted, the shelf life for the toxoid is 30 days.

   Evidence of DTH: Induration > 2 mm.

   Percent Reactors: Up to 75% of immunocompetent persons have cutaneous DTH responses to tetanus toxoid.

   Comments: Immediate reactions (within 30 minutes) may occur in up to 50% of immunocompetent persons. This "wheal and flare" reaction is an allergic response, and may persist for up to two hours. Immediate hypersensitivity does not interfere with the subsequent development of DTH responses (indurations) between 48-72 hours. Systemic side effects, such as fever and anaphylaxis, are rare with tetanus toxoid.

2. CANDIDA ANTIGEN

   DTH reactivity to the Candida antigen is dependent on prior infection with yeast, Candida albicans.

   Antigen: Candida antigen is prepared from sterile culture filtrates of Candida albicans. Some antigens are licensed for diagnostic purposes only, i.e., for testing whether an individual is infected with Candida albicans. Allermed Laboratories, Inc. has a candida skin test (Candin) that is under application to the FDA to be licensed for skin testing. The antigen is known to elicit DTH responses.

   Concentration: Candin skin test antigen currently available by Allermed Laboratories, Inc. for skin testing is supplied in a 1 ml vial (10 doses). Antigens licensed for DTH testing, which become available in the future, should be distributed following the manufacturers' instructions as to the proper dosage.

   Evidence of DTH: Induration >2 mm.

   Percent Reactors: At least 80% of immunocompetent persons will have DTH responses to Candida antigen.

   Comments: Immediate reactions (within 30 minutes) may occur in some individuals. This "wheal and flare" reaction is an allergic response, and may persist for up to two hours. Immediate hypersensitivity does not interfere with subsequent development of DTH responses (indurations) between 48-72 hours. Systemic side effects, such as fever and anaphylaxis, are rare with Candida antigen.

3. MUMPS ANTIGEN

   DTH reactivity to the mumps skin test antigen is dependent on prior disease or immunization with the vaccine. This skin test is useful, since most persons in the United States have been exposed to mumps or vaccinated against the disease.

   Antigen: The mumps skin test antigen is a sterile suspension of killed mumps virus.

   Concentration: The antigen is prepared for skin testing by several companies. It is usually supplied in a 1 ml vial (10 doses).

   Evidence of DTH: Induration >2 mm.

   Percent Reactors: Up to 86% of immunocompetent persons have DTH responses to the mumps skin test antigen.

   CAUTION: This product should not be administered to anyone with a history of hypersensitivity (allergy) to eggs or egg products.

As with other antigens and skin test materials, in rare instances, anaphylactic shock could occur following testing. Epinephrine should be available for such emergencies!

This document is not meant to supersede any existing recommendations, but to simply serve as a "user friendly" guide to understanding anergy and how to test for it.

For additional information concerning anergy testing, contact your local TB Control program or the Division of Tuberculosis Elimination (Mailstop E-10); National Centers for Prevention Services; Centers for Disease Control; Atlanta, GA 30333.

APPENDIX A
WIHS Body Measurement Certifications

Guidelines for all future Body Habitus Certifications/Re-certifications:

1. Sites should ensure they will have six (6) volunteers at the training. Please note these participant volunteers should be scheduled in two waves. Each wave should have three (3) participants; the second wave should be scheduled 1-1/2 hours after the first.
2. Rooms should be ready before the trainer arrives. Gowns, alcohol, and equipment should be set and ready to go and participants should be shown directly to the room in which they will be examined.
3. There can be no more than six (6) clinicians trained on any one day. If more than six clinicians are present when the trainer arrives, she will ask the Project Director to send the extra clinicians home.
4. If it would be helpful, the trainer can provide sites the planned rotation of clinicians/exams in advance. The rotation will have each clinician examining all six patients twice.
5. Each clinician must pass on at least four (4) of the test-retest measures in order to be certified or re-certified. If a clinician only takes five (5) sets of measures, this means they must pass four of the five. Likewise, if they are able to take only four sets of measurements, they must pass all four to be certified.

If you have any questions, please contact Phyllis Tien (ptien@ucsf.edu) or Christine Alden (calden@jhsph.edu) for clarification.

APPENDIX B
Anthropometry Training Manual

TRAINING SPECIFICATIONS

Checklist for equipment and related items needed during training *(Please note: if more than one examiner will be trained, there may be a need for additional exam rooms, measuring tapes, etc.)***:**

__ Exam room with scale, exam table, chair

__ BIA equipment (model 101Q or Quantum II)

__ BIA electrodes

__ NHANES III anthropometry training DVD

__ BIA inservice training video

__ Alcohol wipes

__ Measuring tape (Gulik)

__ Cosmetic marking pencils (e.g., eyeliner make-up pencils)

__ Volunteer participants: ten women (with a minimum of five overweight women)

__ All the examiners to be trained

__ Paper or lightweight cotton gowns

Required number of subjects during training:

Minimum of ten women, with at least five overweight subjects (each examiner should be able to practice the measurements on at least ten women). We recommend scheduling ten to 15 women to ensure that at least ten show up on the day of training.

Required number of subjects to be completed following training:

At least ten subjects during the two weeks following the training.

General Training Schedule

1. Watch training videos (the NHANES III training manual and the BIA inservice training video) and discuss procedures.
2. Trainer demonstrates measurement to examiner on a participant. Examiner practices with the trainer's guidance. Trainer then repeats this demonstration on an overweight participant. Examiners keep practicing on participants until they feel comfortable with the measurements.
3. Trainer evaluates the measurement techniques of all examiners (on a minimum of six women, three overweight).

Training goals

Upon finishing the training, examiners should be able to perform the measurements as following:

Weight: Follow the procedures and measure and record weight for the volunteer. Next, the trainer follows the same procedure and also measures and records weight. Weight should be within 1 lb. between the trainer and the examiner.

- *Circumference:* The examiner follows the procedures and measures and records the circumferences of the volunteer. All marks are removed from the participant. Next, the trainer follows the same

procedures and measures and records the circumferences of the same volunteer. All measurements should be within ± 1 mm between the trainer and the examiner.

- *BIA:* The examiner follows the procedure and measures and records the reactance and resistance. Remove the electrodes and repeat the procedure for a second time with new electrodes. The reactance and resistance measurements should be within ± 1 percent.

Example of a time schedule*		
Time	Activity	Volunteers Needed
9:00am - 10:00am	Watch video with the trainer and discuss procedures: BIA inservice training video and the NHANES III training manual.	None
10:00am - 10:45am	Trainer demonstrates measurement to examiner on a volunteer.	1
10:45am - 12:00pm	Examiners practice with the trainer's guidance. Repeat on an overweight volunteer. Keep practicing on volunteers until examiners feel comfortable with the measurements. Need a minimum of three volunteers, with examiners rotating between volunteers.	Minimum three (two overweight)
12:00pm - 1:00pm	Lunch Break	None
1:00pm - 3:00pm	Continue to practice. Evaluate measurement techniques and record measurements on designated sheets. 1. Weight: Follow the procedures and measure and record weight of the participant. Next, the trainer follows the same procedure and also measures and records weight in lbs. Weight should be within 1 lb. 2. Circumference: Follow the procedures and measure and record the circumferences of the participant. Remove all marks on the participant. Next, the trainer follows the same procedures and measures and records the circumferences and skinfolds of the same participant. All measurements should come within ± 1 mm between the trainer and the examiner. 3. BIA: Follow the procedure and measure and record the reactance and resistance. Remove the electrodes and repeat the procedure for a second time with new electrodes. The reactance and resistance measurements should be within ± 1 percent.	Minimum six (three overweight) Suggest staggering volunteers: e.g., three at 1:00pm and three at 1:45pm

***** This schedule was devised for a site with three examiners. Please adjust your schedule according to your site's needs. For example, if your site has six examiners undergoing the training, it would be better to use additional volunteers to limit the number of persons performing the measurements on one volunteer. Please note that separate examination rooms may be needed for each volunteer since participants may be uncomfortable having body measurements taken with non-clinicians in the same room.

MEASUREMENT INSTRUCTIONS

General Measuring Techniques:

- Participants should remove all clothing, except underwear. Shoes should be removed; thin socks may be worn, if participant desires. Paper or lightweight cotton gowns should be worn during the entire exam.
- Body measurements should always be taken on the right side of the body (unless for a specific reason such as casts or amputations).
- Each site to be measured should be marked with a special marking pencil, such as a eyeliner pencil.
- The measuring tape should be flexible but non-stretchable (i.e., Gulik type).
- Make sure the participant's skin is dry.
- Avoid measuring skinfolds and BIA if the participant is overheated.

Weight

A balance scale should be used, and all weights should be recorded in pounds (LBS). Be sure the scale is balanced so that the indicator is at zero when no weight is on the scale. The scale should be level and on a firm surface (not a carpet). The participant should be instructed to stand in the middle of the platform of the balance scale with head erect and eyes looking straight ahead. Adjust the weight on the indicator until it is balanced. The weight should be recorded in pounds to the nearest 1.0 lb. Please do not make any conversions from kilograms. Have the participant step off the scale, reset the balance to zero and repeat. If measures differ by more than 1.0 lb., repeat a third time. Always record the first measure that most closely matches the third measure. For example, if only two measures were taken (i.e. the first and second measures were within 1.0 lb. of one another) record the first measure taken. If three measures were taken and the second and the third are within 1.0 lb. of each other, record the second measure. If three measures were taken and the first and the third measure were within 1.0 lb. of each other, record the first measure.

Circumference Measurements

Upper Arm Girth: Have the participant stand erect with feet together and the right arm flexed 90E at the elbow with the palm facing up. The examiner is positioned behind the participant. Using a tape measure, mark a point halfway between the lateral projection of the acromian process of the scapula (bump on the backside of shoulder) and the interior part of the olecranon process (elbow). Next, the participant stands with the right elbow relaxed so that the right arm hangs freely to the side. The examiner stands facing the participant's right side. The measuring tape is placed around the upper arm at the marked point perpendicular to the long axis of the upper arm. The tape is held so that the zero end is held below the measurement value. The tape rests on the skin surface, but is not pulled tight enough to compress the skin. The arm circumference is recorded to the nearest 0.1 cm.

Chest Girth: The chest girth is measured at the level of the fourth costo-sternal joints, which laterally corresponds to the level of the sixth ribs. The fourth costo-sternal joint can be located by a two-handed palpitation method whereby the examiner places both the index fingers on the superior surfaces of the clavicles, while the thumbs locate the first intercostal space. The index fingers then replace the thumbs, which are lowered to the second intercostal space. This procedure can then be repeated until the fourth ribs are located. The fourth rib and their costal cartilages are followed medially to their articulations at the sternum, and this point is marked. The participant should be standing with the feet at shoulder width. The arms are slightly away from the body to allow placing the tape around the chest. The measuring tape should be placed horizontally at the marked point. Once the tape is in place, the arms can be lowered to their regular position. Take the measurement at the end of a normal expiration. The chest girth is recorded to the nearest 0.1 cm.

Waist Girth: The study participant is in a standing position. The participant is asked to hold up her gown. The examiner stands behind the participant and palpates the hip area for the right iliac crest (see **Appendix C-1**). The examiner marks a horizontal line at the high point of the iliac crest and then crosses the line to indicate the midaxillary line of the body. The pants and underclothing of the participant must be lowered slightly for the examiner to palpate directly on the hip area for the iliac crest. The examiner then stands on the participant's right side and places the measuring tape around the trunk in a horizontal plane at this level marked on the right side of the trunk. Make sure that the tape is parallel to the floor and that the tape is snug, but does not compress the skin. The measurement is made at minimal respiration to the nearest 0.1 cm.

Hip Girth: The study participant stands erect with feet together and weight evenly distributed on both feet. The participant is holding up the examination gown. If the participant has folds of abdominal fat (pannus) that interfere with the ability to accurately measure the hip circumference, have participant lift pannus up during measurement. **Do not include pannus in measurement.** The examiner places the measuring tape around the buttocks. The tape is placed at the maximum extension of the buttocks (see **Appendix C-2**). The examiner then adjusts the sides of the tape and checks the front and sides so that the plane of the tape is horizontal. The zero end of the tape is held under the measurement value. The tape is held snugly but not tight. The examiner takes the measurement from the right side and records to the nearest 0.1 cm.

Thigh Girth: First, have the participant sitting with her right knee bent at a 90° angle. Mark the nearest border of the patella (knee cap). A measuring tape is placed at the superior aspect of the inguinal crease, which is easily located if the hips are in the sitting position. No pressure is to be applied at inguinal crease; however, folds of fat tissue may have to be lifted on some obese participants to measure at the crease. The exam gown should be lifted. The tape is extended along the midline of the thigh to the line just proximal to the patella (see **Appendix C-3**). The examiner should make a mark (+) at the midpoint of the thigh with a cosmetic marker. Next, have the participant stand with her right leg just in front of her left leg and her weight shifted back from her left leg. This instruction should be demonstrated by the examiner. The edge of the examining table may be used for the participant to hold onto to maintain her balance. The examiner stands on the participant's right side and the measuring tape is placed around the midthigh at the marked point. The tape is positioned perpendicular to the long axis of the thigh with the zero end of the tape held below the measurement value. The tape rests firmly on the skin without compressing the skin. The thigh circumference is measured to the nearest 0.1 cm.

Bioelectrical Impedance Analysis (BIA):

General instructions:

- ***BIA should not be done on pregnant women, on women who are overheated (as indicated by high body temperature) or on those women who have a cardiac pacemaker or who have amputations other than fingers or toes.***
- There should be no portable electrical heater or other electronic device in use in the exam room and the exam table should be non-conductive.
- The battery should be kept current and the equipment should be calibrated weekly.

Procedures:

1. The participant should remove her right shoe and sock. If, for some reason, the procedure must be done on the left side, then make note of it and on subsequent visits always use the left side.
2. The subject should lie on her back, without a pillow, on the exam table, with her arm 30 degrees from her body and thighs not touching.
3. Remove jewelry on the electrode sites.

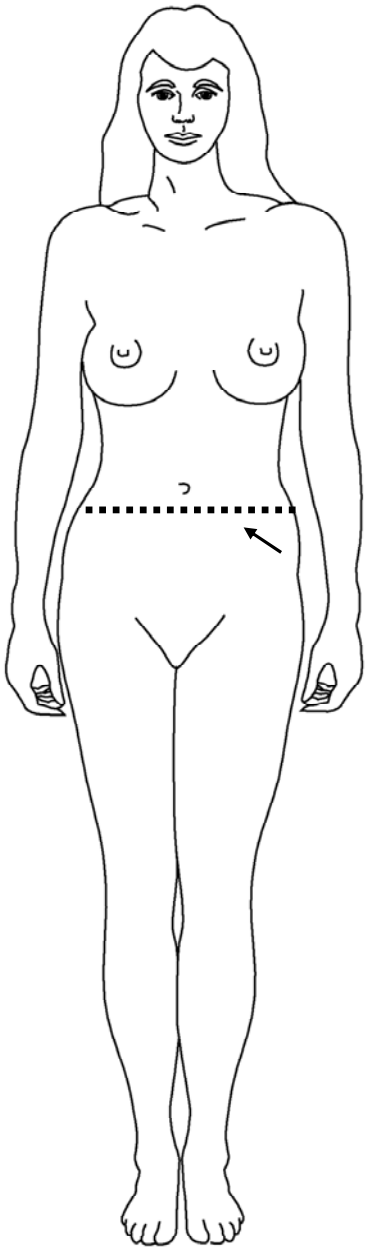
4. The sites where you will place the electrodes should be gently cleaned with an alcohol wipe, particularly if the skin is moist or covered with lotion. Allow alcohol to evaporate before placing electrodes.
5. Attach the electrodes (use whole electrode pads only) and patient cables as described below and as shown in the photographs provided by RJL in the BIA equipment manual. Attach the lead wires to the electrodes with the red leads attached to the wrist and ankle and the black leads attached to the hand and foot. In each case, the red alligator clip should be proximal and the black clip distal.

    - *Right wrist:* Draw an imaginary line on the dorsal surface bisecting the styloid processes of the ulna and radius. Place the center of the electrode along the middle of the imaginary line, and with the tab of the electrode facing out (away from the body).
    - *Right hand:* Place the electrode below the knuckle and above the base of the middle finger, with the tab of the electrode facing out.
    - *Right ankle:* Draw an imaginary line on the dorsal surface of the foot bisecting the medial and lateral malleoli of the ankle. Place the center of the electrode along the middle of the imaginary line with the tab of the electrode facing out.
    - *Right foot:* Place the electrode at least four to five centimeters away from the electrode on the ankle, below the base of the second toe, with the tab of the electrode facing out.

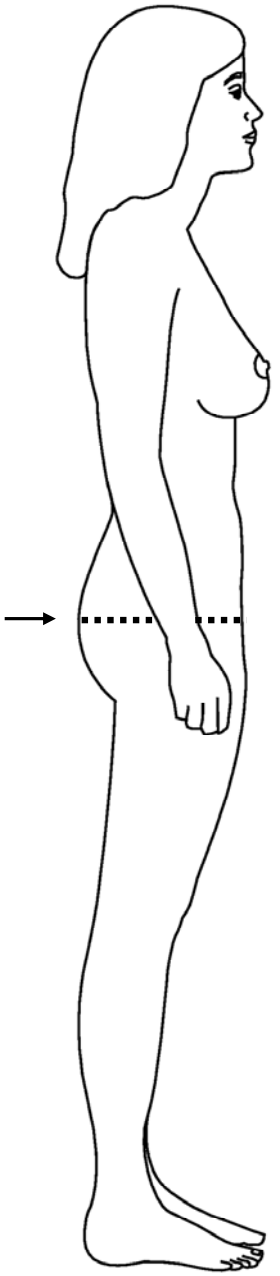
    Attach the lead wires to the electrodes with the red leads attached to the wrist and ankle and the black leads attached to the hand and foot.
6. The participant should remain motionless and relaxed with her arms and legs slightly apart, never touching any other part of the body. The arms should be bent slightly at the elbow with palms down. In cases where the participant's arms and legs cannot be properly spread (because the participant's body is large), the procedure should still be completed and a note made in the comments section. As long as there is no skin contact (the paper gown can be used to separate the arms from the trunk or the legs from each other), no interference with the proper flow of the current should take place.
7. Turn on the analyzer and when the measurements have stabilized, read and record the displayed Resistance (R) and Reactance (Xc). If you were unable to obtain the reading for either of these two measures, enter "-9" for the respective measure.
8. Turn off the analyzer. Remove the electrodes and repeat the entire procedure for a second time. If you were unable to obtain the reading for either of these two measures, enter "-9" for the respective measure.
9. Unhook the leads and remove and dispose of the electrodes. Do not reuse the electrodes.

APPENDIX C
Medical Exam

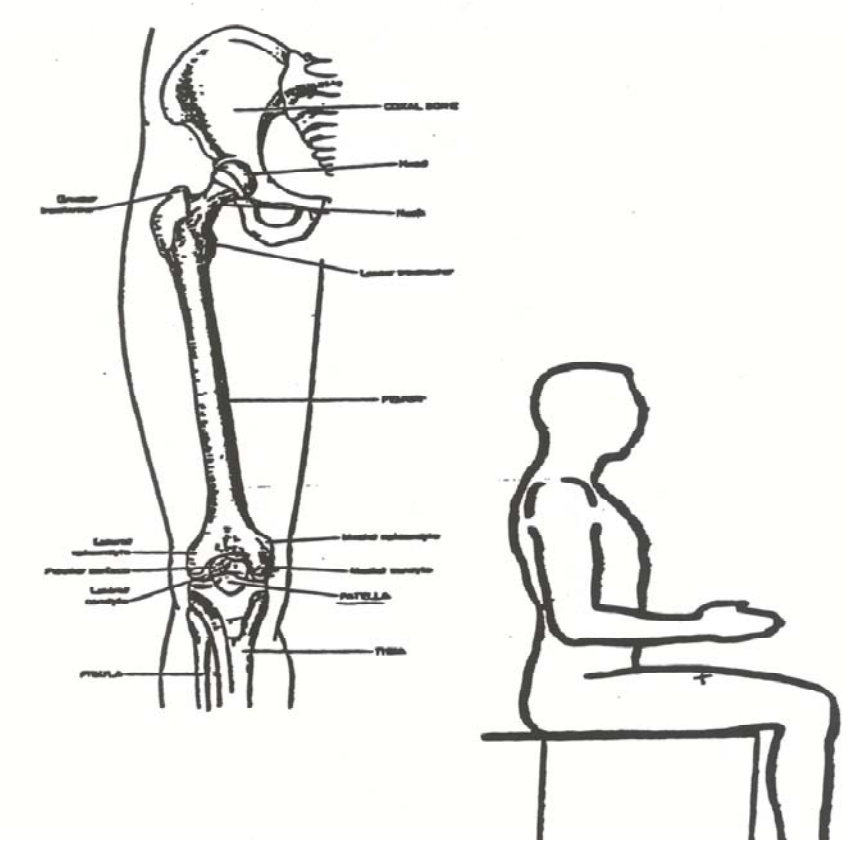
APPENDIX C-1. Measuring tape position for abdominal (waist) circumference



APPENDIX C-2. Measuring tape position for hips (buttocks) circumference



APPENDIX C-3. Participant position for thigh girth measurement



APPENDIX D
Anthropometry Training Form

PARTICIPANT'S INITIALS: ____ ____ ____ **TEST** (Circle)**: 1 2**

EXAMINER: _____ **DATE:_____**

BODY MEASURES (cms):

Site	Trial 1	Trial 2	Trial 3	Average Measure
Upper Arm				
Breast/Chest				
Waist				
Hip				
Thigh				

BIA RESULTS (ohms):

	Reactance	Resistance
Measurement 1		
Measurement 2		

Comments:

APPENDIX E
UAB Cytopatholgy Requisition

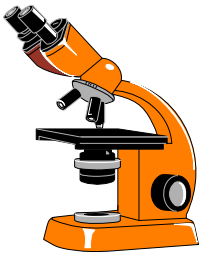
(USE BLACK OR BLUE BALL POINT PEN ONLY)

PATIENT ID		STUDY SITE	COLLECTION DATE
	UAB MEDICINE CYTOPATHOLOGY REQUISITION-NIH WIHS 508 20th Street South HSB 100 (205) 934-2025 FAX (205) 975-7056		

SPECIMEN ID	AGE	SEX	DATE OF BIRTH mm/dd/year	CLINICIAN NAME	PHYSICIAN Name/ #
		F			Strickler, H MD # TBD

LAB USE ONLY

Did you label specimen?



TEST AND SOURCE FOR GYNECOLOGIC SPECIMEN SUBMITTED

TEST

- ☐ Conventional Smear
- ☐ Other

SOURCE

- ☐ Cervical/Endocervical
- ☐ Vaginal
- ☐ Other _____

CLINICAL INFORMATION AND HISTORY

LMP _____

- ☐ Routine
- ☐ Abnormal Pap smear
- ☐ Hormone Replacement Therapy
- ☐ Post Menopausal
- ☐ Hysterectomy
- ☐ Pregnant _____ wks
- ☐ Post Partum _____ wks

Relevant clinical history

_____

_____

_____

_____

The Pap test is only a screening procedure to aid in the detection of cervical cancer and its precursor lesions. It should not be used as the sole means in the detection of cervical cancer. False-negative and false-positive results may occur.