WOMEN'S INTERAGENCY HIV STUDY

SECTION 10: LABORATORY SPECIMEN COLLECTION AND PROCESSING PROCEDURES

I. OVERVIEW OF SPECIMEN COLLECTION AND IDENTIFICATION

A variety of specimens will be obtained from each participant enrolled in the WIHS. Not all specimens are collected at every visit. See **MOO Sections 5** and **6** for details on baseline visits for 1994/95, 2001/02, and 2011/12 and WIHS-V cohorts. Specimens will be collected by phlebotomy and during the Medical Exam. Required laboratory tests are listed in **Appendix B, Schedule of Laboratory Evaluations**. See **Section I.C** for the **Specimens Charts** for details on collection, processing and storage of specimens. These charts include the test name, type of test, and, where applicable, specimen storage and shipping information.

A. DESIGNATION OF LAB TESTS AND SPECIMENS

Of the laboratory specimens obtained, some will be processed immediately and others will be stored for future testing. Where tests are performed will vary as well. Categorized in relation to timing and location, each laboratory test and its requisite specimen is designated as one of the following:

1. *Local Immediate* – process immediately through your institution's laboratory, e.g., CBC/Diff and T-cells.
2. *Central Immediate* – ship overnight to a central lab for processing, e.g., Pap smears.
3. *Local Save & Batch* – process and prepare for local storage (as part of a local repository).
4. *Central Save & Batch* – process and prepare for *temporary* storage locally, e.g., lipids. Some central save and batch specimens will be shipped to a central laboratory every one to three months for testing; others will be shipped periodically to the central repository.
5. *Exam Site* – process and prepare immediately for testing in the clinic. Results are recorded on the *Gynecological Exam Form* (*F08*), e.g., KOH and saline preps.

B. IDENTIFICATION OF SPECIMENS

1. Labels are to be used on each *specimen* (except glass slides).
2. WIHSID, specimen date, specimen code and visit number must be recorded on the label. This information is essential for future WIHS staff, testing personnel, and the central repository. WIHSIDs for 2001/02 recruits are distinguishable from those of 1994/95 recruits in that the second digit for all 2001/02 recruits is a "2." WIHSIDs for 2011/12 recruits are distinguishable from those of other recruits in that the second digit for all 2011/12 recruits is a "3." WIHSIDs for WIHS-V recruits are distinguishable in that the first and second digits represent the clinic number, and the third digit (i.e., "4") represents the recruitment wave.
3. Identify the source and type of specimen by using the numeric specimen code, or s-code, listed in **Appendix A, Standardized WIHS Specimen Codes, Volumes, and LDMS Codes**.
4. Attach labels to specimens at the time of collection and *prior* to transportation to the lab.
5. To ensure labels are attached securely to cryovials and collection tubes, polyester protective tape (liquid nitrogen safe) may be placed over the labels and wrapped securely around the tubes before freezing specimens or transporting.
6. WIHSID, visit, s-code, and date are to be recorded in pencil directly on the frosted end of glass slides that are collected for WIHS tests.

C. SPECIMENS CHARTS

If a participant is not able to provide all tubes, be sure to collect the lavender-tops for CBC/Diff and T-Cell count. The draw order was modified at visit 20 to comply with recommendations from publications, manufacturers, and laboratory standards organizations regarding pre-analytical variability contributed by additives in blood collection tubes. 2011/12 or WIHS-V recruits at the baseline visit should not contribute to Special Event (SE) or MSK additional collections.

1. BLOOD, IN ORDER OF PRIORITY:

TEST AND PROTOCOL	COLLECTION	COLLECTED VOLUME	LAB TYPE	PROCESSING☼	STORAGE	SHIPMENT	COMMENTS
1. HIV Antibody ELISA C R	Red-top. (HIV- participants, all new recruits). May be collected at screening visit for new recruits.	1-2ml Minimum	Local Immediate	Site Specific	None	NA	Perform test locally. Not required after baseline visit on HIV+.
2. Western Blot C R		Site Specific	Local Immediate	Site Specific	None	NA	Record WB bands starting with visit 9.
3. Liver/Renal Function Partial Chemistries § C R	Red-top, Tiger-top or Gold SST	2-5 ml (minimum allowed by local lab)	Local Immediate	Gently invert 5 times. Allow to clot 30 minutes in vertical position. Centrifuge within 6 hours at 400 x g for 10 minutes.	None	NA	Chemistry tests include: ALT, AST, BUN, Alk phos., Albumin, BUN, Creatinine, GGT, Bilirubin, Calcium, Phosphate†
4. Lipid Panel and Insulin ‡ C R	Tiger-top or Gold SST (plastic) even visits only. Insulin at even, fasting visits only.	8.5 ml	Quest Diagnostics (Baltimore)	Gently invert 5 times. Process within 1 hour after drawing blood.	Freeze aliquots at -80°C (+/- 10°) within 8 hours	Ship to QD monthly on dry ice.	Collect at specific visits per CVD protocol. 1 ml aliquots.
5. Serum for Central Repository C	Red-top, Tiger-top or Gold SST all participants at follow-up visits.	8.5 ml		Gently invert 5 times. Allow to clot 30 minutes in vertical position. Centrifuge at room temp within 1 hour at 1100-1300 x g for 15 minutes.		Ship to Central Repository on dry ice.	3x1 ml + 1x1.8ml aliquots only to Central Repository.
6. Hepatitis B & C Serology R	Tiger-top or Gold SST (plastic) baseline only.	4 ml	Local Immediate	See *#3*.	None	NA	
7. RPR Syphilis § R	Tiger-top or Gold SST (plastic) baseline only.	4 ml	Local Immediate	See *#3*.	None	NA	
8. Serum for Central Repository R	5 Tiger-top or Gold SSTs (plastic).	3 x 8.5 ml, 2 x 4 ml		See *#5*.		Ship to Central Repository on dry ice.	

TEST AND PROTOCOL	COLLECTION	COLLECTED VOLUME	LAB TYPE	PROCESSING☼	STORAGE	SHIPMENT	COMMENTS
9. Serum from Special Event (SE) for Central Repository	**Red-top, Tiger-top or Gold SST from SE-eligible.**	**8.5 ml**		**See *#5*.**		**Ship to Central Repository on dry ice.**	**1 ml aliquots only to Central Repository.**
10. Serum for Musculoskeletal (MSK) substudy	**Red-top, Tiger-top or Gold SST from MSK-eligible.**	**8.5 ml**		**See *#5*.**		**Ship to Central Repository on dry ice.**	**1.2 ml aliquots only to Central Repository.**
11. Plasma Repository [C R]	**5 CPT 8 ml each from all participants.**	**Total 40 ml**	**Central and Local Repository**	**Gently invert 8 times. Process within 6 hours under sterile conditions. Centrifuge CPT within 6 hours of draw for 20 minutes at 1500 x g. Separate cells from plasma within 27 hours; preferably within 6 hours.**	**-80°C (+/-10°) 1. Central 3x1 ml + 1x1.8ml plasma 2. Balance to local**	**Ship to Central Repository on dry ice.**	**4-5 x 1 ml, 2-3 x 0.5 ml for 2011/12 & WIHS-V new recruits.**
12. Viable Cells (PBMC) & Dry Cell Pellets [C R]					**-80°C (+/-10°) ♦ 1. Central Viable Cells 2. Central Dry Cell Pellets 3. Balance to local**		**3-4 vials of viable cells with DMSO at 1x10E7 cells/ml. 4 vials of pellets with PBS at 5x10E5 cells/ml.**
13. Viable Cells (PBMC) for special event	**3 CPT 8 ml each from SE-eligible.**	**24 ml**	**Central Repository**	**See *#11-12*.**	**-80°C (+/-10°) ♦ 1. Central Viable Cells**	**Ship to Central Repository on dry ice.**	**2-3 Viable cells with DMSO at 1x10E7 cells/ml**
14. Viable Cells (PBMC) for MSK	**3 CPT 8 ml each from MSK-eligible.**	**24 ml**	**Central Repository**	**See *#11-12*.**	**-80° C (+/-10°) ♦ 1. Central Viable Cells**	**Ship to Central Repository on dry ice.**	**3 Viable cells with DMSO at 1x10E7 cells/ml**
15. CBC with Differential and platelets * [C]	**Lavender (Collect on HIV- at even follow-up visits beginning with visit 11).**	**2.5 ml or Site specific**	**Local Immediate**	**Gently invert 8 times. Process CBC and Differential within 24 hours after drawing blood.**	**None**	**NA**	**Minimum 3-part automated differential is required.**
16. T-Cell count and subsets (CD4, CD8) * [C]	**Lavender (Collect on HIV- at baseline visit only beginning with visit 36).**	**2.5 ml**	**Local Immediate (Within 24-30 hours of draw)**	**Site Specific**	**None**	**N/A**	**Run on specimen drawn at same time as CBC. Perform at a DAIDS IQA-certified Lab (see NOTE below)**
17. HIV RNA for TaqMan [C]	**Lavender/PPT (HIV+ only).**	**5-6 ml**	**Local Batch**	**Gently invert 8 times. Spin lavender for 10 min at ≤1300 x g, aliquot, repeat spin. Spin PPT once only for 10 min at 1000 x g.∞**	**Aliquots should be stored at -80°C (+/-10°).**	**Send a minimum of 1.1 ml for testing.**	**Perform at a DAIDS VQA-certified Lab (see NOTE below)**

TEST AND PROTOCOL	COLLECTION	COLLECTED VOLUME	LAB TYPE	PROCESSING☼	STORAGE	SHIPMENT	COMMENTS
18. Hemoglobin A1C ‡ C	**Lavender (Even visits only).**	**2.5 ml or Site specific**	**Quest Diagnostics (Baltimore)**	**Gently invert 8 times. Process within 30 hours after drawing blood.**	**Aliquots should be stored at -80°C (+/-10°) within 30 hours of blood collection.**	**Ship to QD monthly on dry ice.**	**Collect at specific visits per CVD protocol. 1 ml aliquots.**
19. Glucose ‡ C	**Gray-top (Even visits, fasting only).**	**2.5 ml or Site specific**	**Quest Diagnostics (Baltimore)**	**Gently invert 8 times. Process within 30 hours after drawing blood. Spin for 10 min at 800-1000 x and aliquot plasma.**	**Aliquots should be stored at -80°C (+/-10°) within 30 hours of blood collection.**	**Ship to QD monthly on dry ice.**	**Collect at specific visits per CVD protocol. 1 ml aliquots.**

* CBC/Diff and T-cell count may be performed from the same tube.

∞ Lavenders should be processed within 6 hours. A PPT should be spun within 2 hours and plasma taken off within 24 hours of collection. Keep at room temperature until ready to freeze for long-term storage.

♦ Dry cell pellets to be stored at -80°C (+/-10°). Viable cells with cryomedium to be stored at -80°C (+/-10°) for no longer than 30 days and then shipped to the Central Repository for long term storage at -150°C.

† Phosphate may not be included in the standard panel for all WIHS sites.

☼ The manufacturer of blood collection tubes recommends a certain number of inversions for different tubes. An inversion is one complete turn of the wrist, 180 degrees, and back.

‡ The *fasting metabolic panel* includes: total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), calculated low-density lipoprotein cholesterol (LDL-C), triglycerides (TRIG), and insulin. The *non-fasting metabolic panel* includes: TC, HDL-C, and direct LDL-C. Hemoglobin A1C can be performed regardless of fasting status. Glucose can only be performed on fasting gray-top plasma. Collection of all these specimens was switched to even-numbered visits beginning with visit 36.

§ Liver/Renal Function Partial Chemistries and RPR Syphilis may be performed on same tube at the baseline recruitment visit.

C Collected and aliquoted for the Core follow-up visit protocol.

R Collected and aliquoted for baseline visit of 2011/12 and WIHS-V recruits.

NOTE: Should any WIHS site choose a specimen processing laboratory that is not already approved for participation in the DAIDS Virology or Immunology Quality Assurance Programs, the PI must contact the WIHS Program Official named in the Notice of Award in order to initiate the approval process. Participation in these programs is not automatic and the addition of new labs has to be pre-approved by the official who oversees the VQA and IQA contract resources at DAIDS.

Special Event definitions:

- **Elite controller –** At core visit prior to current visit participant had undetectable viral load and was not on antiretrovirals.
- **ART-naïve at first ART/HAART visit –** Participant was ART-naïve at all visits prior to current visit and has started ART/HAART since her last visit.
- **Seroconverter –** Participant was identified as an HIV seroconverter based on HIV test at her last core visit.

2. URINE SPECIMENS:

TEST AND PROTOCOL	COLLECTION AND PRIORITY	VOLUME	LAB TYPE	PROCESSING	STORAGE	SHIPMENT	COMMENTS
1. Urine Pregnancy Test	1st priority Clean catch, mid-stream in sterile container. Transfer remainder to transport conical tube with screw cap.	Site Specific	Exam site	None	None	None	Pregnancy Test Kits do not need to be standard across sites.
2. Urinalysis (micro and macro)	2nd priority for new recruits at baseline. Clean catch, mid-stream in sterile container. Send to local lab or transfer to UATT.	8 ml	Local immediate	Test within 2 hours if sample not in a special UATT.	Store at ambient or refrigerate until testing; UATT can be stored at ambient.	None	If sample not processed within 2 hours, then store in UATT for testing within 72 hours. Record results on L10.
3. Urine for Renal Tests	3rd priority for all new recruits at baseline.	2 x 1 ml	Central Save & Batch	Process within 4-6 hours prior to centrifugation for supernatant and pellet. Aliquot whole urine in 2 x 1 ml vials.	Store at -80ºC (+/-10°) until shipped	Ship to Central Repository on dry ice	If sample not processed within 4-6 hours then store at 4ºC.
4. Urine Supernatant and Pellet	2nd priority Even and baseline visits only. Priority 4 for all new recruits at baseline.	5 ml	Central Save & Batch	Process within 4-6 hours. Centrifuge at 1000 x g for 10 minutes. Aliquot scheme dependent upon visit number. Place in cryovial and add PBS to resuspend pellet.	Store at -80ºC (+/-10°) until shipped	Ship to Central Repository on dry ice	If sample not processed within 4-6 hours then store at 4ºC.

3. VAGINAL SPECIMENS:

TEST AND PROTOCOL	COLLECTION AND PRIORITY	VOLUME	LAB TYPE	PROCESSING	STORAGE	SHIPMENT	COMMENTS
1. pH	1st priority Dacron Swab.	N/A	Exam Site	Collect spec from posterior and lateral vaginal fornices + Apply to pH paper.	None	None	Record result on GYN Exam Form (F08)
2. Trichomonas Culture	2nd priority Sterile Dacron swab.	N/A. Obtain sample from posterior vaginal fornix	Exam site	Place swab in warm (room temperature) Diamond's Media; incubate at 35-37°C.	Store in incubator x 7 days	N/A	Examine 1 drop of broth at high power x 400 after days 2-3 and 6-7 of incubation

TEST AND PROTOCOL	COLLECTION AND PRIORITY	VOLUME	LAB TYPE	PROCESSING	STORAGE	SHIPMENT	COMMENTS
3. Bacterial Vaginosis	3rd priority Sterile Dacron swab.	N/A	Central Save & Batch	Air dry after collection. Save & batch for future testing.	Room temperature in slide holders	Ship to central repository at the end of the visit	
4. *T. vaginalis* (Wet Mount)	4th priority Sterile Dacron swab.	N/A	Exam Site	Normal Saline prep. Microscopic evaluation after exam.	None	None	Record result on GYN Exam Form (F08)
5. Microscopic Evaluation (KOH Prep)				10% KOH prep. Microscopic evaluation after exam.			
6. Amine (Odor) Test				Note odor immediately after adding KOH to prep slide.			

4. CERVICAL SPECIMENS:

TEST AND PROTOCOL	COLLECTION AND PRIORITY	VOLUME	LAB TYPE	PROCESSING	STORAGE	SHIPMENT	COMMENTS
1. Pap Smear	5th priority Spatula and Cytobrush or spatula and cotton-tipped applicator, if patient is pregnant.	N/A	Central Immediate	Use one glass slide.	None	Ship Mon –Wed using traceable courier to UAB at room temperature	Pap smear kits purchased by WIHS site. Instructions for shipping in Section V.
2. Gonorrhea and Chlamydia	6th priority for new recruits at baseline. Consult local testing facility.	N/A	Local immediate	Local test methodologies differ, consult with testing facility.	None	None	Record results on L09 and L13
3. HPV DNA swab	6th priority, Priority 7 for new recruits at baseline. Sterile Dacron swab.	N/A	Central Save and Batch	Process within 48 hours of collection. Vortex and aliquot to 1.8ml cryovial.	Store at -80ºC (+/-10°) within 72 hours of collection	Ship to Central Repository on dry ice	Kit provided by Digene. Use Digene DTM only.

5. CERVICAL-VAGINAL LAVAGE:

TEST AND PROTOCOL	COLLECTION AND PRIORITY	VOLUME	LAB TYPE	PROCESSING	STORAGE	SHIPMENT	COMMENTS
1. HPV by hybrid capture	Collect in 1x15ml conical tube and aliquot in the lab as follows: 1st & 2nd priority.	3 ml	Central	None	In 1.5 ml aliquots -80°C (+/-10°)	Ship to Central Repository on dry ice	
2. HPV by PCR	3rd priority.	1.5 ml					
3. Repository	4th priority.	1.5 ml					
4. Repository	5th + priority.	Remainder	Local repository			N/A	

6. COLPOSCOPY AND BIOPSY:

TEST AND PROTOCOL	COLLECTION AND PRIORITY	VOLUME	LAB TYPE	PROCESSING	STORAGE	SHIPMENT	COMMENTS
1. Colposcopy and Biopsy	Standard procedure Colposcopy/Biopsy as indicated.	N/A	Biopsies Local	Site Specific	Site Specific	N/A	

7. CENTRAL AND LOCAL REPOSITORY PRIORITIES FOR BLOOD:

	PRIORITY ONE: WIHS CORE	PRIORITY TWO: LOCAL REPOSITORY	PRIORITY THREE: OTHER STUDIES
1. Serum	3 x 1 ml/vial + 1 x 1.8 ml/vial to Central *New Recruits:* 6-10 x 1 ml, 9-13 x 0.5 ml	1 x 1 ml or remainder to Local *New Recruits:* 6 x 1 ml, 9 x 0.5 ml	*SE:* 2-4 x 1 ml *MSK:* 2-7 x 1.2 ml
2. Plasma (CPT)	3 x 1 ml/vial + 1 x 1.8ml/vial to Central *New Recruits:* 4-5 x 1 ml, 2-3 x 0.5 ml	2 x 1 ml or remainder to Local *New Recruits:* 4 x 1 ml, 2 x 0.5 ml	N/A
3. Viable PBMC (CPT)	3-4 vials at 1x10E7 cells/ml in DMSO to Central (viable cells) ♦	Remainder at 1x10E7 cells/ml in DMSO to Local (viable cells) ♦	*SE:* 3 vials at 1x10E7 cells/ml in DMSO *MSK:* 3 vials at 1x10E7 cells/ml in DMSO
4. Dry Cell Pellets (CPT)	4 vials of 5x10E5 cells/ml in PBS to Central ♦	Remainder at 5x10E5 cells/ml in PBS to Local ♦	N/A

8. SUMMARY OF EXPECTED CENTRAL REPOSITORY ALIQUOTS:

Fill as many vials as possible to the stated volume or concentration. Overfilling up to 0.2 ml, but not exceeding the limit of the vial, to account for later discrepancies in volume due to long-term storage is acceptable. Ensure that the manifest and vial volumes are in agreement.

	S-CODE†	FOLLOW-UP VISITS	BASELINE 2011/12 & WIHS-V NEW RECRUITS
1. Serum	**1, 3, 42**	**3 vials x 1 ml each + 1 vial x 1.8 ml**	**2-4 vials x 1 ml, 12 vials x 0.5 ml**
a. New recruit baseline visit reserved testing	**113, 114, 115**	**N/A**	**6 vials x 1 ml, 1 vial x 0.5 ml**
b. SE**	**1, 3, 42**	**2-4 vials x 1 ml each**	**N/A**
b. MSK	**117**	**2-7 vials x 1.2 ml each**	**N/A**
2. CPT Plasma	**4**	**3 vials x 1 ml each + 1 vial x 1.8 ml**	**4-5 vials x 1 ml, 2-3 vials x 0.5 ml**
3. PBMCs	**6**	**3-4 vials at 1x10E7 cells/ml each**	**3-4 vials at 1x10E7 cells/ml each**
b. SE**	**6**	**2-3 vials at 1x10E7 cells/ml each**	**N/A**
b. MSK	**118**	**3 vials at 1x10E7 cells/ml each**	**N/A**
4. Dry cell pellets	**10**	**4 vials at 5x10E5 cells/ml each**	**4-8 vials at 5x10E5 cells/ml each**
5. Cervical-vaginal lavage	**25**	***Follow-up visits 1&2:* 7 vials x 1 ml each** ***Follow-up visits 3+:* 4 vials x 1.5 ml each**	**7 vials x 1 ml each**
6. Cervical swab supernatant	**112**	**1 vial x 0.6-1 ml**	**1 vial x 0.6-1 ml**
7. New recruit baseline visit whole urine	**116**	**N/A**	**2 vials x 1 ml each**
8. Urine supernatant ‡	**12**	***Visits 36, 40, 44:* 2 vials x 1 ml + 1 vial x 1.5 ml** ***Visits 38, 42, 46:* 1 vial x 1 ml + 1 vial x 1.5 ml**	**3-4 vials x 1 ml each**
9. Urine pellet with PBS ‡	**111**	***Visits 38, 42, 46:* 1 vial**	**1 vial**

† Specimen codes depend on fasting status, collection method, and reserved test designation. ‡ Even-numbered visits only.
**** SE = SPECIAL EVENT; see page 4 for definitions**

9. TOTAL WHOLE BLOOD COLLECTED VOLUME:

Study and Tube Type	Core Follow-up		Baseline 2011/12 & WIHS-V	
Core Red-top or Gold or Tiger-top SSTs	HIV+: 10.5 – 13.5 ml	HIV-: 11 – 15.5 ml	HIV+: 42 – 42.5 ml	HIV-: 43 – 44.5 ml
Core Lavender (EDTA) tubes (HIV+)	10 –11 ml		10 - 12 ml	
Core Lavender (EDTA) tubes (HIV-)	Even visit: 2.5 ml	Odd visit: 0 ml	5–6 ml	
Core CPTs	40 ml		40 ml	
Fasting metabolic (SST, EDTA, NaFl)	Even visit: 13.5 ml	Odd visit: 0 ml	13.5 – 15.5 ml	
Non-fasting metabolic (SST, EDTA)	Even visit: 11.5 ml	Odd visit: 0 ml	11 – 12.5 ml	
SE** CPTs and serum*	32.5 ml		N/A	
MSK CPTs and serum* (Visits 35-42)	32.5 ml		N/A	
Total possible	**40.5 – 112.5 ml**		**88.5 – 113 ml**	

* Eligibility for these studies is exclusive; participants should NOT contribute to both at the same visit.

II. BLOOD SPECIMENS

A. BLOOD DRAWING PROTOCOL

1. PURPOSE

   - To ensure the most efficient use of the volume of blood that is available for participants enrolled in the study.
   - To establish a set of priorities for phlebotomists and laboratory technicians in the event that insufficient blood is obtained for all studies.
   - To designate samples for the central and local repositories.

2. BACKGROUND

   A multicenter cooperative study requires a standardized but flexible protocol for prioritizing the use of limited samples. The order of blood draw has been standardized by national and international authorities because of the possibility of cross contamination between tubes due to different additives. WIHS staff are generally able to collect all required tubes for their participants. Laboratory quality improvement plans have significantly reduced analytic error so that "preanalytical variability now represents the most important source of errors that can lead to inaccurate patient results."[i] Additionally, the Clinical and Laboratory Standards Institute (formerly known as the National Committee for Clinical Laboratory Standards or NCCLS) specifies the following order for a blood draw[ii, iii, iv]:

   1. Coagulation tube (e.g., blue closure or sodium citrate additive)
   2. Serum tube (with/without clot activator or gel separator)
   3. Heparin tube (with/without gel separator) (e.g., green closure)
   4. EDTA tube (lavender closure precedes white or pink closures)
   5. Glycolytic inhibitor (e.g., gray closure)

   If the phlebotomist cannot complete the entire blood draw, it is important that the priority of collection is as follows:

   a. EDTA – anticoagulated whole blood for CBC/Diff, T-cell subsets, and HIV RNA.
   b. Plasma and cells for central storage.
   c. A modest volume for the local and central repositories.
   d. The remainder of the studies required by the protocol.
   e. Additional specimens for the local and central repositories.

NOTE: It is preferred that when aliquoting serum or plasma for shipment to the central repository, vials are filled to the stated volumes. In the event that all aliquotted vials will not have the full volume, do not distribute the total volume evenly among all vials. Fill as many vials as possible to the stated volume. It is more important to have correct volumes in fewer vials than to have less volume in more vials.

Phlebotomy should be performed before the interview unless the participant reports that she is not fasting or if the Physical Exam will be done at a different visit. When the visit needs to be split, blood should be drawn at the time of the Physical Exam. If a participant is menstruating, less than eight weeks post-partum, or post-termination of a pregnancy, the Gynecological Exam should be postponed to another time within a two-week completion window if the woman is menstruating, or until after eight weeks if the woman is post-partum or post-termination of pregnancy. However, blood should be drawn at the time of the Physical Exam. See **MOO Section 7** for exceptions to this rule.

B. HIV TESTING REQUIREMENTS

1. All 1994/95 WIHS recruits were required to be HIV tested at screening or enrollment. HIV antibody ELISA was required for all 1994/95 recruits.
2. 2001/02 recruits who report that they are HIV-positive and can provide hard-copy documentation of a positive ELISA and Western Blot were not required to be re-tested for HIV. All self-reporting HIV-negative women were tested for HIV.
3. 2011/12 and WIHS-V recruits should be screened for study entry in a two-stage process; a screening and then a baseline visit. HIV antibody testing is required if the recruit reports being HIV-negative or if the recruit reports being HIV-positive but does not have hard-copy documentation of a positive ELISA and Western Blot. When possible, antibody testing should be performed at the screening visit to reduce phlebotomy required at the baseline visit. For recruits that require testing, sites may draw blood for HIV antibody testing at the screening or baseline visit according to the following criteria:
    a. For women who test HIV-infected at the screening visit, baseline labs may exclude the HIV antibody test.
    b. For women who test HIV-uninfected at the screening visit and who have their baseline visit within 14 days of their screening visit, the baseline labs may exclude the HIV antibody test.
    c. For women who test HIV-uninfected at the screening visit and who have their baseline visit more than 14 days after their screening visit, the baseline labs will include the HIV antibody test.
    d. For women who did not have an HIV antibody test at the screening visit, the baseline labs will include an HIV antibody test.
4. HIV Antibody ELISA is to be repeated at six-month intervals on women who tested negative for the HIV antibody at their previous visit or developed an indeterminate status after the enrollment visit.
5. Positive HIV ELISA tests must be confirmed by Western Blot. Bands will be reported for all tests performed starting at visit 9.

C. MISSING AND REPEAT LAB TEST REQUIREMENTS

1. HIV testing is required at follow-up visits for all HIV-negative participants – if missing, it will need to be drawn again within the two-week visit window or performed from an aliquot in the repository.
2. 1.1 ml of save and batch serum will be designated for back-up HIV testing.
3. CBC/Diff and Flow Cytometry are required to be from the same blood draw event. If one or both are missing, sites are to make every effort to have the participant return within the two-week window for blood draw, or, in the case of unreliable results, redraw. These tests cannot be run on specimens that have been frozen. Beginning with visit 11, CBC/Diff will be performed on HIV-negatives only at even-numbered visits. For visits 11 through 35, Flow Cytometry was performed on HIV-seronegative women only at even-numbered visits. Beginning with visit 36, Flow Cytometry will be performed only at the baseline visit for HIV-negative women.
4. Apollo is programmed not to allow “missing” test results for these three tests only. Forms will need to be data entered with the reason for missing results when applicable.
5. Missing labs or unreliable test results may be drawn again within a two-week window of the visit.

D. COLLECTION METHODS AND PROCEDURES

The participant should be made as comfortable as possible before commencing phlebotomy since a large volume of blood will be obtained. Precise and quality technique is necessary to preserve the participants' veins and well-being. Phlebotomy should be performed using aseptic technique. Phlebotomy steps are as follows:

A. Preparation:

1. The phlebotomist should wash her/his hands and **wear gloves throughout the procedure.** It is strongly recommended that the phlebotomist wear a lab coat and mucous membrane protection. To prepare for venous blood collection and reduce participant burden, phlebotomists should assemble tubes in the order stated in **Section II.E.**
2. Identify yourself to the participant and positively identify the participant's WIHSID and date of birth.
3. The participant should rest for fifteen to thirty minutes prior to the blood draw. If a participant lies on her back and sits upright just prior to the draw this could alter the quality of the specimens collected and accuracy of results.[v] If the participant cannot sit for blood collection, she should be in the same position for her blood draw at all study visits.
4. Ensure the participant is comfortable and that her arm is supported. Observe Standard Precautions during the procedure. Ask the participant to make a fist or hold the gripper provided.
5. Apply the tourniquet to the participant's arm two inches above the site selected for venipuncture. Use only the tightness necessary for vein visualization; an excessively tight tourniquet will cause stasis of the blood and will give incorrect laboratory results.
6. If a satisfactory vein is identified in the antecubital area, remove the tourniquet and proceed with aseptic technique. It is preferable to remove the tourniquet while preparing the alcohol, gauze, and tubes since the tourniquet will be on for such a long time.
7. Once the tubes, alcohol, and gauze have been assembled, reapply the tourniquet, and disinfect the venipuncture site (and any of the phlebotomist's fingers that might come in contact with it). Working outwards in a circular motion, wipe the site selected with alcohol and then wipe dry with gauze pad.

B. Venipuncture[vi]:

1. Inform the participant you are about to stick her and that she will feel a sting. At the same time, use firm pressure and insert the needle into the selected vein. DO NOT JAB. Fill tubes with blood in the order listed in **Section II.E.**

   The tourniquet should be removed as soon as blood starts to flow – this avoids an increase in lipid values due to prolonged venous occlusion. If the blood flow slows or stops, try pulling the needle back slightly or advancing it in slightly. Make sure that you do not exit the vein – this will cause an unsightly and painful hematoma.

   <u>It is important to completely fill Cell Preparation Tubes (CPTs)</u> (8 ml capacity) when drawing blood. Because CPTs contain anticoagulant, they must be completely filled, otherwise plasma will be diluted.

2. After all tubes have been filled, withdraw the needle carefully – avoid jabbing the participant. Under-filled or over-filled tubes can result in an incorrect blood/additive ratio and thus impact clotting times. Apply sterile gauze to the venipuncture site. Ask the participant to apply firm pressure to the site.
3. Inspect the venipuncture site. If bleeding has stopped, apply a fresh piece of gauze and tape.
4. WIHS "Re-Stick" Policy: When a participant comes for a visit and the technician is unsuccessful in getting blood on the initial venipuncture, the tech will ask the participant for permission to attempt collection at another site. If the second venipuncture is unsuccessful, the tech will ask the participant for permission to have a third attempt made by another tech. Technicians are limited to two sticks per participant. If the blood draw is unsuccessful and the participant does not grant permission for a repeat stick, a clinician must be notified before the participant leaves the clinic.
5. WIHS Participant Emergency Policy:
    - If a participant states that she may faint before/during/after blood drawing:
        - Place the participant in a wheelchair and escort the participant to a stretcher holding area.
        - Place the participant in a bed with one side rail up and collect the blood specimen.
        - After blood is drawn, put up the side rail and check the participant's condition. The participant should be stable and well enough to move before allowing her to leave the clinic.
        - If the participant continues to feel faint while lying down (10 minutes after blood collection), check the participant's pulse and blood pressure. Contact a clinician to inform her of the participant's condition and location.
    - If a participant faints:
        - Move the participant to a sitting position or a bed so she does not fall on the floor or otherwise injure herself.
        - Notify a senior clinician so she can check the participant's pulse and blood pressure.
        - A senior clinician must monitor the participant and decide upon further action based on the participant's well being.
    - If a participant faints during the blood draw:
        - Remove the needle and place gauze on the phlebotomy site. Remove the tourniquet from the participant's arm.
        - Proceed with instructions for when a participant faints.

C. Processing:

1. Immediately after finishing venipuncture, label the tubes. The following information will need to be recorded on the label by the phlebotomist:
    - WIHSID
    - Specimen (venipuncture) Date
    - Study Visit Number
2. Gently invert all tubes several times after they have been completely filled.
3. Discard the needle and any other used supplies in appropriate containers. Clean the work area and prepare for the next participant.
4. Place the tiger-top SST and red-top tubes in a vertical position for 30 minutes to allow the clotting process to complete.

5. Complete the WIHS *Specimen Collection Form* (*F09* or *F29r* for baseline, *F29* for follow-up visits) and the *Repository Specimen Processing Form* (*L20*). Make sure the WIHSID number, Specimen Date, Specimen Time, and Study Visit Number are included on the forms. Please note that *F29, F29a, F29r, CV29* and *F31* are to be completed by the site clinician/phlebotomist. There is no question that cannot be answered by site personnel; therefore, the laboratory should not complete the specimen collection forms. Laboratory personnel should only complete *L20*.
6. If blood cannot be delivered to the lab within six hours of the draw, place red-top tubes in the refrigerator until the courier arrives. CPTs are to be centrifuged at 1500 x g for 20 minutes, then stored and transported in a horizontal position at room temperature. Tiger-top SSTs are to be centrifuged at 1100 x g for 15 minutes in a horizontal rotor within six hours of collection. WIHS laboratories should not process grossly hemolysed specimens. Samples should not be processed if the serum or plasma is indistinguishable from the blood clot. Labs should report back to the clinic who will coordinate a repeat blood draw. Labs are not required to enter hemolysis in the LDMS.
7. Record the time CPTs were centrifuged on the *F29, F29r,* and *L20*, as appropriate.
8. Forward the pink copy only of *F09* or a Xeroxed copy of *F29* or *F29r* to the laboratory with the blood specimens.

E. BLOOD SPECIMEN DESIGNATIONS

Proper organization, packaging, shipping and handling of human blood borne pathogens insure sample integrity while maintaining the timely and safe transfer of specimens. Specific packaging and shipping procedures and staff training must be followed in accordance with federal regulations. Always ship by overnight carrier. Check with your facility as to which carrier to use and for the specific labeling requirements for biohazardous shipments. IATA regulations require that the sender notify the recipient of the dangerous goods prior to shipment. This is to alert the receiving party that the shipment is coming, and to ensure that prior arrangements have been made for someone to receive the shipment at delivery time. When contacting the recipient, include the courier company name, air bill number, and the date of expected delivery. Refer to **Appendix A** for standardized use of all specimen and volume codes on all aliquots described below.

1. Tests slated for **Local Immediate** processing are as follows:
   a. *HIV Antibody ELISA and Western Blot* (when applicable) (1-2 ml red-top). All HIV-negative participants at follow-up and 2011/12 and WIHS-V recruits at screening or baseline visit.
   b. *T-Cell Count & Subsets* (2-5 ml Lavender). Baseline only for HIV-negatives, all 2011/12 and WIHS-V recruits at baseline. Local flow labs may do both CBC/Diff and T-cells on the same tube.
   c. *CBC with Diff & Platelets* (3-5 ml Lavender). Even visits only for HIV-negative, all 2011/12 and WIHS-V recruits at baseline.
   d. *Liver/Renal Function Chemistries* (2-5 ml SST) at all visits beginning with visit 14.
   e. *RPR Syphilis* (2 ml SST) at baseline visit for 2011/12 and WIHS-V recruits. Local labs may be able to use serum from the Liver/Renal Function Chemistries sample.
2. Samples slated for **Central Save & Batch** testing:
   a. *HIV RNA quantitation* (5-6 ml Lavender). For HIV-positive only. Sites locally save and batch samples for testing periodically during the visit.
   b. *Lipid Panel and Insulin* to Quest, Baltimore (serum). Even visits only beginning with visit 36. All 2011/12 and WIHS-V recruits at baseline.

c. *Glucose* to Quest, Baltimore (plasma). Even visits only beginning with visit 36. All 2011/12 and WIHS-V recruits at baseline.
d. *Hemoglobin A1C* to Quest, Baltimore (EDTA whole blood). Even visits only beginning with visit 36. All 2011/12 and WIHS-V recruits at baseline.

3. Samples slated for **Local and Central (Repository) Save & Batch**:
   a. *Plasma and Cell Repository*
      i. At follow-up core visits, process 40 ml of whole blood from CPTs. Plasma and cells should be aliquotted as follows: Minimum of 3x1 ml + 1x1.8 ml of plasma for central and the remainder for local repositories. From same CPTs above: Place 3-4 at 1x10E7 cells per ml with DMSO for viable cell aliquots and 4x5E5 cells per ml with PBS for cell pellet aliquots to the Central Repository. Any remaining plasma, viable cell, or cell pellet aliquots can be sent to the local repository at the discretion of each site.
      ii. At baseline visits for 2011/12 and WIHS-V recruits, process CPTs into aliquots of plasma, cells, and cell pellets as follows: 4-5 x 1 ml and 2-3 x 0.5 ml of plasma, 4 at 1x10E7 cells per ml in DMSO of viable cells, and 8 x 5E5 cells per ml with PBS of cell pellets.
      iii. At follow-up core visits, process an additional 30 ml of whole blood from CPTs if participant is eligible for a Special Event (SE) draw. Pool with five core CPTs and ensure that minimum requirements for core viable cells and cell pellets are met first. Aliquot 2-3 at 1x10E7 cells per ml in DMSO of viable cells with core specimen codes.
      iv. At follow-up core visits, process an additional 30 ml of whole blood from CPTs if participant is MSK-eligible at baseline substudy visit (35-38) and follow-up substudy visit (39-42). Pool with five core CPTs and ensure that minimum requirements for core viable cells and cell pellets are met first. Aliquot 3 at 1x10E7 cells per ml in DMSO of viable cells with reserved specimen code.
   b. *Serum Repository:*
      i. At follow-up visits, process 10 ml whole blood from red-top or serum-separator tubes and aliquot serum as follows: 3x1 ml + 1x1.8 ml for central and 2x1 ml for local repositories. A 10 ml tiger-top Serum Separator Tube (SST) draws only 8.3 ml of whole blood as the tiger-top SST contains a gel barrier (approximately 1.5 ml). After centrifuging, the gel separates serum from the blood clot and prevents leakage of RBC and WBC contents into the serum. Phlebotomists should collect blood in more than one tiger-top SST since testing will require a full 10 ml of whole blood. Collect 1x10 ml red-top or 2x10 ml tiger-top SSTs (with gel barrier). These will be aliquotted and stored at the central repository for future testing.
      ii. At baseline visits for 2011/12 and WIHS-V recruits, process serum collection tubes into aliquots of serum as follows: 6-10 x 1 ml and 9-13 x 0.5 ml. A minimum of 4 x 1 ml has been designated for HCV antibody testing, HCV viral load, and HCV genotype; 2 x 1 ml for sex steroids; 1 x 0.5 ml for hsCRP. Appropriate s-codes should be used in order to facilitate later identification of reserved aliquots in the repository.
      iii. At follow-up core visits, process an additional 8.5 ml of whole blood from serum collection tube if participant is SE-eligible. Aliquot in 2-4 x 1 ml with core specimen codes.
      iv. At follow-up core visits, process an additional 8.5 ml of whole blood from serum collection tube if participant is MSK-eligible at baseline substudy visit (35-38) or

follow-up substudy visit (39-42). Aliquot in 2-7 x 1.2 ml with reserved specimen code.

4. Laboratory Designations by Test:

Exam Site	N/A
Local Immediate	• HIV Antibody ELISA & Western Blot for HIV-negatives, all 2011/12 and WIHS-V recruits • T-cell count subsets, at baseline only for HIV-negatives, all 2011/12 and WIHS-V recruits at baseline • CBC and diff/platelets, at even visits only for HIV-negatives, all 2011/12 and WIHS-V recruits at baseline • Liver/renal function chemistries • Hepatitis B & C serology at baseline for 2011/12 and WIHS-V recruits • RPR Syphilis at baseline for 2011/12 recruits and WIHS-V
Local Save & Batch	• Local repository aliquot scheme for cells, serum, plasma is at the discretion of each site once central repository requirements have been satisfied
Central Save & Batch	• Serum to repository 3 x 1 ml + 1 x 1.8 ml at follow-up; 6-10 x 1 ml and 9-13 x 0.5 ml at baseline for 2011/12 and WIHS-V recruits • Viable cells to repository 3-4 at 1x10E7 cells per ml with DMSO • Dry cell pellets to repository 4 x 5E5 with PBS • CPT Plasma to repository 3 x 1 ml + 1 x 1.8 ml at follow-up; 4-5 x 1 ml and 2-3 x 0.5 ml at baseline for 2011/12 and WIHS-V recruits • Lipid Panel, Insulin, Hemoglobin A1C, Glucose to Quest at even visits only and at baseline for 2011/12 and WIHS-V recruits • HIV RNA quantitation to Quest Diagnostics or Rush lab • Serum in 2-4 x 1 ml and Viable cells in 3 at 1x10E7 with DMOS to repository for SE-eligible • Serum in 2-7 x 1.2 ml and Viable cells in 3 at 1x10E7 with DMSO to repository for MSK-eligible

F. SPECIMEN TRACKING

Labs are to keep a record of shipments and samples received from clinics. All samples of different types or drawing times should receive unique identifiers. See **MOO Section 31** for instructions on standardized specimen labeling for samples going to the central repository.

The database or other records should document the:

- unique identifiers
- date & time received
- WIHSID
- S-code
- name (if applicable)
- date & time of specimen collection from the *F29* or *F29r*; date & time of processing and freezing from *L20*
- type of collection tube (SST, EDTA, CPT, urine, vaginal, oral, etc.)
- volume of liquid specimens
- time of centrifugation
- location in freezers or laboratory

G. PROCESSING OF PLASMA AND CELLS FROM CELL PREPARATION TUBES (CPTs)

1. COLLECTION

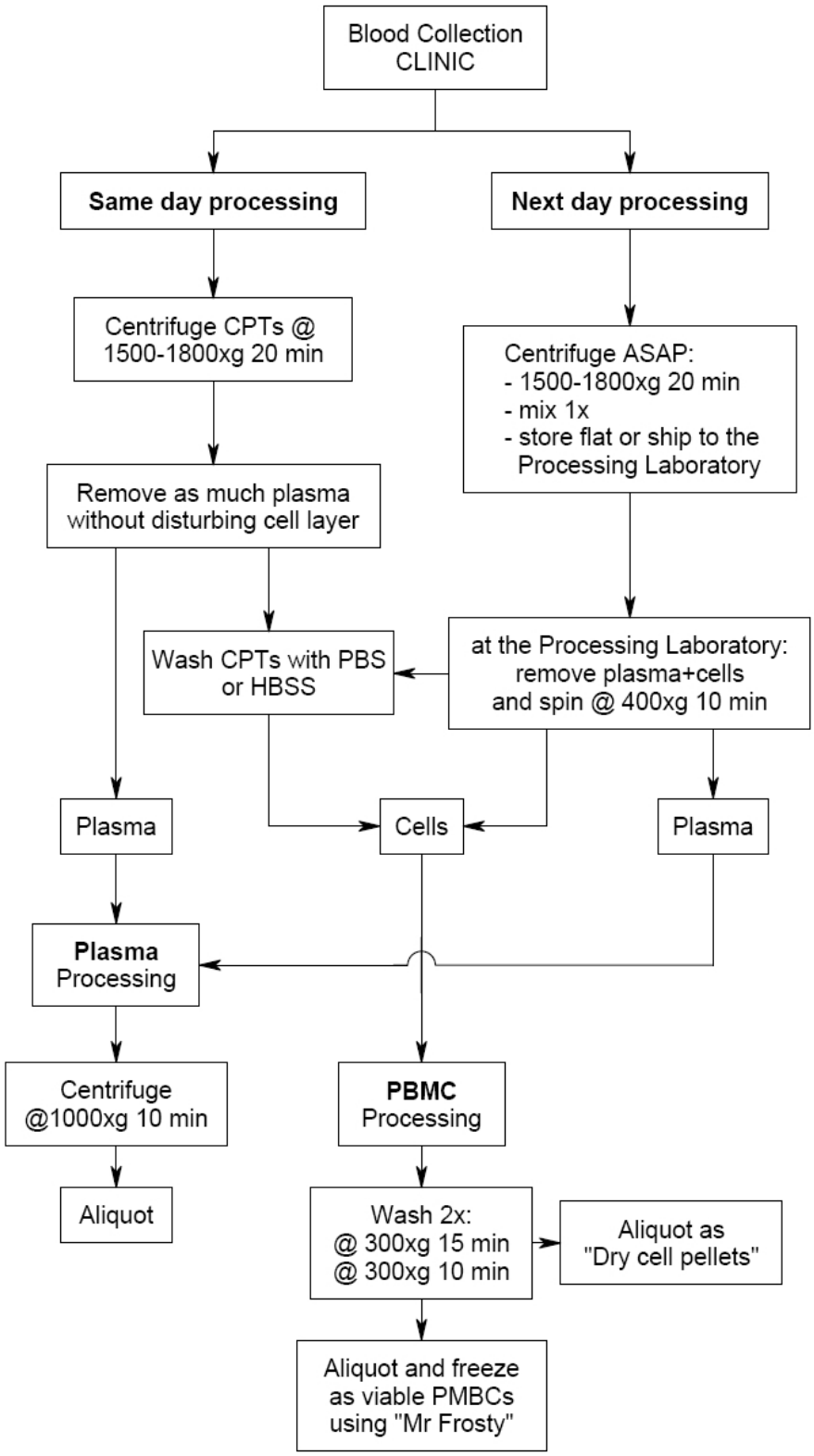
Use 8 ml Cell Preparation Tubes (CPTs) (Sodium Citrate VACUTAINER brand tubes, Becton-Dickinson Reorder # 362761). Each 8 ml tube will yield about 3–4 ml of plasma. From a normal donor, using an 8 ml CPT, one can recover 10–12 million mononuclear cells (approximately 85% lymphocytes, 14% monocytes and 1% granulocytes). From HIV+ individuals, the number is lower (4–8 million). CPT tubes with less than 1 ml of blood should not be processed.

2. PROCESSING CPTs[vii]

   This protocol describes procedures for initial centrifugation to stabilize CPTs for next day processing, as well as steps for processing CPTs immediately (See **Figure 1**). There are several general principles of CPT processing that should be familiar to all WIHS staff handling these tubes:

   - CPTs should be kept at room temperature and centrifuged within six hours of blood collection.
   - If plasma separation and cell freezing will take place the day after specimen collection, then CPTs must be centrifuged within six hours and inverted once. This ensures that cells are suspended in plasma in the tube until the plasma and cells can be separated and frozen.
   - To prevent loss of cells and tube damage, do not exceed 1800 x g.
   - Sites should only use centrifuges with swing-out rotors.
   - Technicians should be familiar with the Boyum cell extraction method.[viii]
   - Ensure that the correct RPM setting on the centrifuge is used based on the stated G-force in this protocol. Re-measure the radius and re-calculate the RPM anytime the centrifuge, rotor, or bucket is changed. [ix]

Figure 1. CPT Processing Flow



A. CPT PREPARATION FOR NEXT DAY CELL SEPARATION AND FREEZING
   1. Within 6 hours of collection, centrifuge CPTs at 1500-1800 x g for 20 minutes at room temperature.
   2. Invert each CPT once, after centrifugation, to mix plasma and cells.
   3. For overnight storage and shipment, place CPTs flat on their side.
   4. Record the time of centrifugation on *F29* or *F29r* for CPTs centrifuged but not processed within six hours. WIHS laboratory technicians should complete *L20*.

B. CPT PROCESSING AT THE LABORATORY
   1. PLASMA SEPARATION
      a. Allow tubes to reach room temperature prior to centrifugation.
      b. If tubes arrive unspun, centrifuge CPTs in a swing-out rotor at 1500-1800 x g for 20 minutes at room temperature.
         i. Carefully inspect the centrifuged CPT to be sure the gel barrier is evenly distributed across the full width of the tube. Mononuclear cells and platelets will be in a whitish layer just under the plasma layer. CPTs should look like the "After Centrifugation" tube pictured in **Figure 2**.
         ii. Gently transport the CPT to the Laminar flow hood without disturbing the mononuclear cell layer.
         iii. Using a sterile transfer pipette, aspirate as much plasma as possible without disturbing the cell layer. Each tube should yield approximately 3-4 ml plasma. Combine aspirated plasma from all tubes and distributed equally into two 15 ml tubes.
         iv. Centrifuge the plasma layer at 1000 x g for 10 minutes to remove any contaminating PBMC and platelets. Set cells aside for further processing.

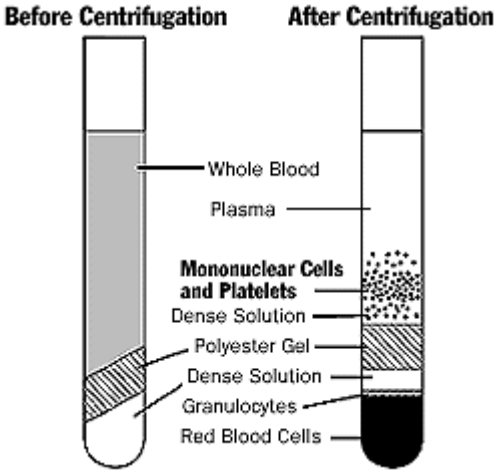


Figure 2. Distribution of Cellular Fractions in CPTs Before and After Centrifugation

      c. If tubes arrive pre-spun, carefully remove the CPT stopper. Transfer the entire plasma and cell layer above the gel to conical tube and spin at 400 x g for 10 minutes.
         i. Remove plasma to another conical and spin at 1000 x g for 10 minutes. Set cells aside for further processing.
      d. Aliquot the plasma as follows and store at -80°C (+/-10°) for no more than one month before shipping specimens to the central repository:
         i. 3 x 1 ml aliquots + 1 x 1.8 ml aliquot (central repository)
         ii. Remainder (local repository) site-specific aliquot volumes

2. PBMCs EXTRACTION
    a. While the plasma is spinning add sterile PBS[x] to each CPT to fill the tube. (Hanks' Balanced Salt Solution, HBSS, can also be used whenever PBS is mentioned.) Recap each tube. Be careful not to mix caps if processing multiple participants at the same time. Mix cells by inverting tubes five times, or using a pipette. Be sure to collect any remaining cells trapped on the stopper or on top of the gel by gently washing and inverting.
        i. Carefully remove the CPT stopper and pipette the mononuclear cell suspension from each tube into a single, sterile, 50 ml, conical, screw-top centrifuge tube or multiple 15 ml tubes.
        ii. Centrifuge the tube(s) with PBMCs at 300 x g for 15 minutes.
    b. Aspirate supernatant but leave 1 ml or less in the conical. Resuspend cells by gently vortexing or tapping the tip of the conical tube. (It is much easier to resuspend the pellet when it is at the bottom of the conical before adding PBS. Cells are likely to form a clump if PBS is added. It is easier to resuspend a semi-dry pellet rather than repeatedly vortex a pellet to disturb clumps.)
    c. Add 10 ml PBS. Re-cap and mix cells by inverting tube(s) five times.
    d. Centrifuge tube(s) for 10 minutes at 300 x g. Aspirate as much supernatant as possible without disturbing cell pellet. Resuspend cells by gently vortexing or tapping tube with your index finger.
    e. Add 10 ml of PBS. Recap tube(s). Mix cells well by inverting five times.
3. DETERMINING CELL CONCENTRATION
    a. HEMACYTOMETER
        i. Take 10µL (microliters) of cells and mix with an equal volume of Evans or Trypan Blue and count in a hemacytometer. You may have to dilute this more if the cell concentration is high and hard to read.
        ii. Determine how many cells per ml you have in the sample using the cell count obtained with a hemacytometer and multiply by total cell volume.[xi]
    b. AUTOMATED CELL COUNTER

        You may also use an automated cell counter to determine cell concentration in accordance with your laboratory's procedures. [xii]
4. DRY CELL PELLETS

    Once the total number of cells has been determined, transfer a maximum of four million cells into four microfuge tubes (i.e., 4 cryovials x 5E5 cells per ml). Add 0.5 ml PBS to each tube. Microfuge these tubes for five minutes and carefully aspirate off the PBS (10 microliters of PBS can stay above the pellet) without disturbing the cell pellet. Pellets should be stored at -80°C (+/-10°).
5. CRYOPRESERVATION OF PBMCS: STEPS AFTER DETERMINING CELL CONCENTRATION
    a. Centrifuge tube(s) with PBMCs again for 10 minutes at 400 x g.
    b. Remove and discard the supernatant.
    c. Resuspend cells by gently vortexing or tapping tube.
    d. Place tube(s) with cells on ice.
    e. Add cold cryoprotective medium in a drop-wise fashion, with constant mixing, over one to two minutes. Add enough cryopreservation medium to have PBMC concentration of 1x10E7 per 1 ml (10 million cells per ml).[xiii]

f. Dispense 1 ml aliquots of the cell suspension into pre-cooled (4˚C) cryovials. Four aliquots should be sent to the central repository and any remaining aliquots should be stored locally.

g. Immediately place the cryovials in the "Mr. Frosty" container and follow instructions printed on each "Mr. Frosty".[xiv] The "Mr. Frosty" container must be at approximately the same temperature as cryovials at the beginning of the freezing process to ensure the correct rate of freezing (~1°C/min). For example, if cells are on ice, then the "Mr. Frosty" should already be at 4°C. Processing technicians should be familiar with the manufacturer's instructions for the "Mr. Frosty" and Nalge Nunc vials.

h. Place "Mr. Frosty" container(s) in the bottom of a -80°C (+/-10°) freezer for 16–72 hours, then transfer cryovials to a -80°C (+/-10°) freezer for storage. Aliquots for local storage should be transferred to a -150°C freezer for long-term storage.

i. Viable cells for the central repository should be stored for no longer than 30 days after the blood draw and shipped on dry ice. Sites should attempt to fill as many boxes as possible with cells in a 30-day period. It is acceptable to ship at least one partially full box in the shipment.

H. PROCESSING SERUM FROM RED-TOP TUBES

1. Blood should be collected aseptically in a red-top tube.
2. Allow the blood to clot for 30 minutes in a vertical position and centrifuge at 400 x g for 10 minutes.
3. Aliquot serum into 1.8 ml labeled cryotubes (3 x 1 ml, plus 1 x 1.8 ml). Label all specimens according to protocol and ensure correct fasting vs. non-fasting specimen codes. Keep remainder locally until results from local immediate and central save & batch tests are confirmed, and then discard.
4. Discard the clot.
5. Freeze at -80°C (+/-10°).

I. PROCESSING SERUM FROM GOLD OR TIGER-TOP SERUM SEPARATOR TUBES (SST)

1. Blood should be collected aseptically in a gold or tiger-top SST.
2. Gently invert the tube approximately five times immediately after collection to activate the clotting process. Place the tube in a vertical position for 30 minutes to allow the clotting process to complete.
3. Filled SSTs should be kept at room temperature and centrifuged within six hours of collection. SSTs must be centrifuged at room temperature in a horizontal rotor (swing-out head) at 1100 x g for 15 minutes.
4. Serum contained within the spun SST can be "poured off" into a serum transport vial for transit to the processing lab. (This cuts down on the volume of biohazardous material that has to be transported to the processing lab.) Alternatively, the whole, spun SST can be transported to the processing lab if the clinic does not want to deal with open biohazard tubes.
5. When the ambient temperature (temperature outside of the clinic) is above 75° F, the samples should be transported to the processing lab on -20°C frozen cold packs. When using frozen cold packs, make sure that the tubes do not come in direct contact with the frozen cold packs, as this may cause local freezing of the blood tube. Transport blood to the processing lab at optimal temperatures: ambient to cool.

6. Observe correct specimen code usage for fasting vs. non-fasting samples that are used for the metabolic panel (see **Appendix A**).
7. Aliquot serum into 1.8 ml labeled cryotubes (3 x 1 ml, plus 1 x 1.8 ml). Label all specimens according to protocol and ensure correct fasting vs. non-fasting specimen codes. Keep remainder locally until results from local immediate and central save & batch tests are confirmed, and then discard.
8. Freeze at -80°C (+/-10°).

J. PROCESSING PLASMA FROM GRAY-TOP TUBES (for glucose testing)

1. Blood should be collected aseptically in a 3 ml sodium fluoride/potassium oxalate gray-top tube. Tubes should be filled completely, if possible.
2. Gently invert the tube approximately five times immediately after blood draw to adequately mix the anticoagulant and the blood.
3. Tubes do not need to be centrifuged immediately, but instead can be transported overnight as whole blood to another lab for processing. If tubes are to be shipped to a processing lab, ship as described in **J.5**.
4. Centrifuge with a horizontal rotor (swing-out head) at 1100 x g for 15 minutes.
5. Aliquot plasma in 1 ml amounts to 1.8 ml labeled cryotubes. Label all specimens according to protocol and ensure correct fasting vs. non-fasting specimen codes. Keep remainder locally until result is confirmed, and then discard.
6. Freeze at -80°C (+/-10°).

K. PROCESSING EDTA PLASMA (LAVENDER TUBES) (for HIV RNA)

1. After aseptic collection of whole blood in the lavender top tube, gently invert the tube 8 - 10 times. Tubes should be filled completely, if possible.
2. After mixing, store the tube upright at room temperature until centrifugation. For best results, centrifuge within six hours of blood collection.
3. Centrifuge at room temperature at $\leq$1,300 x g for 10 minutes.
4. Remove the BD Hemogard™ Closure and aliquot plasma into a separate vial using a transfer pipette.
5. Centrifuge a second time at room temperature at $\leq$1,300 x g for 10 minutes.
6. Transfer plasma using a pipette to the final aliquot tube. Keep on ice or refrigeration temperature prior to freezing.
7. Aliquot at least 1.1 ml of plasma to 1.8 ml labeled cryotubes. Keep remainder locally until result is confirmed and then discard.
8. Freeze plasma in aliquots within six hours of collection at -80°C (+/-10°).

L. PROCESSING EDTA PLASMA (PPT) (for HIV RNA)

1. After aseptic collection of whole blood in the PPT, gently invert the tube 8 - 10 times. Tubes should be filled completely, if possible.
2. After mixing, store the tube upright at room temperature until centrifugation. For best results, centrifuge within two hours of blood collection.
3. Centrifuge at room temperature at 1,100 x g for a minimum of 10 minutes.
4. To obtain an undiluted plasma sample, remove the BD Hemogard™ Closure and aliquot plasma into a separate vial using a transfer pipette. Be sure NOT to disturb the barrier with the tip of the pipette. Maintain separated plasma at refrigeration temperature prior to freezing.

5. Aliquot at least 1.1 ml of plasma to 1.8 ml labeled cryotube. Keep remainder locally until result is confirmed and then discard.
6. Freeze plasma in aliquots within six hours of collection at -80°C (+/-10°).

M. PROCESSING TUBES FOR CARDIOVASCULAR DISEASE SUB STUDY

Please see **MOO Section 30** for specific instructions on collection, processing, and storage of specimens for the CVD substudy. Fasting specimens for CVD include preceding specimens in **J** and **K**.

N. PROCESSING TUBES FOR MUSCULOSKELETAL SUB STUDY

Please see **MOO Section 37** for specific instructions on eligibility and study design. If eligible, participants will be asked to contribute three additional CPTs and one additional 8.5 ml SST. Samples should be collected and processed in the same manner as core WIHS CPTs and SSTs. Labs should pool and count all cells from CPTs to create 3-4 aliquots of viable cells at 10 million cells per ml for core, 4 aliquots of dry cell pellets at 500,000 cells per ml for core, and then 3 aliquots of viable cells at 10 million cells per ml for MSK. Serum should be placed in 2-7 aliquots at 1.2 mls each. Specimen codes for MSK are listed in **Appendix A**. All samples should be sent to the central repository with core WIHS samples in regular shipments.

O. CRYOPRESERVATION REAGENT PREPARATION

1. CRYOPRESERVATION MATERIALS
   - 8 ml VACUTAINER CPT with Sodium Citrate (Beckton-Dickinson VACUTAINER Cat # 362761)
   - RPMI 1640
   - 200 mM L-glutamine
   - GIBCO PBS 7.4 (Cat # 10010 015)
   - DMSO (tissue culture grade)
   - FBS
   - 50 ml Sterile Polypropylene Conical Plug-Seal Centrifuge Tubes (Fisher Cat # 05-539-6)
   - Trypan Blue
   - 1.8 ml Cryovial
   - Mr Frosty container (Nalge Nunc International)
   - 56° C Water Bath
   - Liquid Nitrogen Freezer
2. CRYOPRESERVATION REAGENT PREPARATION
   a. FBS (Fetal Bovine Serum) – (Flow, Gibco, Biologos, Hazelton)
      i. Thaw the 500 ml bottle of FBS completely.
      ii. Inactivate the FBS by immersing the entire bottle into a 56°C water bath for 30 minutes.
      iii. Aliquot in sterile 45 ml volumes (optional). Each aliquot should be thawed once then used.
      iv. Label the bottle with the day of inactivation and store at –20°C for 18 months from the date of receipt.
   b. Cryopreservative Medium (ACTG): Prepare as needed (reagent good for 24 hours only).
      i. Add 5 ml of DMSO to 45 ml of heat inactivated FBS.
      ii. Mix well by inversion.
      iii. Cool the media to 2.8°C and mix well prior to use.

III. COLLECTION AND PROCESSING OF URINE SPECIMENS

A mid-stream, clean voided specimen for pregnancy test, if indicated, is required at every visit. Urine should be collected in a sterile cup transferred to a screw top conical tube (while wearing PPE and over a sink) for transport to the local lab. The clean catch, mid-stream sample should be reserved for storage in the central repository. At follow-up visits during WIHS IV (i.e., visits 31, 33, and 35), urine was collected at odd-numbered visits for repositing. Beginning with visit 36, urine for repositing will be collected at even-numbered visits. At visits 36, 40, and 44, supernatant only (2 x 1 ml + 1 x 1.5 ml) will be collected. At visits 38, 42, and 46, supernatant (1 x 1 ml + 1 x 1.5 ml) plus pellet (1 vial) will be collected.

At baseline visits for 2011/12 and WIHS-V recruits, whole urine should be sent for local immediate urinalysis, aliquoted for central repository samples, and then centrifuged for supernatant and pellet central repository samples.

A. CLEAN VOID SPECIMEN

1. Perform a pregnancy test for every woman unless she is s/p hysterectomy or bilateral oophorectomy or greater than 50 years old. Record results on the *F31* (follow-up) or *F31r* (baseline) forms.
2. At odd visits 31 through 35 and at even visits 36 through 46, transfer 5 ml of the specimen to a conical tube for immediate processing at the local lab.
3. At baseline visits for 2011/12 and WIHS-V recruits, send 8 ml urine to the testing lab for micro and macro urinalysis within two hours. If the sample will not be tested within two hours, then transfer to a UATT and send to the testing lab within 72 hours.
4. It is preferable that urine for central repository aliquots be processed immediately. Urine can be stored at room temperature for up to 4-6 hours. If the sample will not be processed within 4-6 hours then it should be refrigerated at 4°C until processing.
    i. Aliquot 2 x 1 ml of whole urine into cryovials for future renal testing for 2011/12 and WIHS-V recruits only at the baseline visit.
    ii. Centrifuge conical for 10 minutes at 1,000 x g.
    iii. Place supernatant into two or three cryovials, depending upon visit number.
    iv. At visits 38, 42 and 46, add 70 uL of cold PBS or HBSS to the pellet. Resuspend the pellet and transfer to a cryovial.
    v. Freeze at -80°C (+/-10°).

B. LABORATORY DESIGNATIONS BY TEST

Exam Site	• Urine pregnancy
Local Immediate	• Micro and macro urinalysis for 2011/12 and WIHS-V recruits at baseline
Central Immediate	• N/A
Local Save & Batch	• Local repository aliquot scheme for urine is at the discretion of each site once central repository requirements have been satisfied
Central Save & Batch	• Whole urine for future renal testing for all 2011/12 and WIHS-V recruits at baseline • Urine supernatant at even visits; all 2011/12 and WIHS-V recruits at baseline • Urine pellet at even visits 38, 42, 46; all 2011/12 and WIHS-V recruits at baseline

IV. COLLECTION AND PROCESSING OF GENITAL SPECIMENS OBTAINED DURING PHYSICAL EXAM

A. VAGINAL SWABS

See **MOO Section 9** for the order of specimen collection during baseline and follow-up gynecological examinations. Sample collection for 2011/12 and WIHS-V recruits at baseline is the same as at follow-up visits with the addition of samples for gonorrhea and Chlamydia testing.

1. Swab #1 (Vaginal):

The pH of the posterior vaginal pool is measured using a specimen obtained with a dacron swab from the posterior and lateral vaginal fornices applied to 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. Perform pH evaluation at every visit.

2. Swab #2 (Vaginal): (NOTE: *Trichomonas culture is a site-specific option.)*

A sterile dacron swab is used to sample the posterior vaginal pool for trichomonas culture; after sampling, the swab is placed directly into the Diamond medium that has been warmed to room temperature. Break off the swab shaft and gently agitate the sample.

3. Swab #3 (Vaginal) for Bacterial Vaginosis:

Using a sterile dacron swab, obtain Vaginal Swab #3 from the posterior fornix. Roll swab on a glass slide and allow to air dry. Store at room temperature. Using a lead pencil, write WIHSID on frosted end of glass slide, date specimen obtained, visit #, and "V" to identify the source of the slide as vaginal. All gram stain slides collected through visit 12 have been shipped to the central lab for testing (c/o Lorna Rabe, Magee Women's Hospital Research Center, Pittsburgh, PA). Gram stain slides collected after visit 12 should be placed in plastic slide holders, padded with tissue and stored at Fisher Bioservices at room temperature for future testing. A vaginal swab for Bacterial Vaginosis will be collected at every visit.

4. Swab #4 (Vaginal) for *T. Vaginalis*, 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 each of two glass slides; two drops of saline and of 10% KOH are then applied to one slide each. Immediately after mixing the

specimen with KOH, the slide is placed close to the nose to detect the fishy amine odor; the presence of a fishy odor indicates a positive test. Cover slips are placed over the saline and KOH specimens. Perform saline and KOH prep tests and Amine test at every visit.

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

a. Saline prep:

Perform microscopic examination of the slide looking for Clue Cells (epithelial cells heavily studded with bacteria) and trichomonads. The presence of motile trichomonads indicates a positive test for trichomonas. A wet preparation is considered negative if trichomonads are not seen when entire coverslip area is viewed. Also assess whether there are increased WBCs (i.e., whether WBC:Epithelial cells is greater than 1:1).

b. Vaginal KOH prep:

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

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

B. CERVICO-VAGINAL LAVAGE

Cervical/vaginal lavage is performed on all women whether or not the cervix is present. At the baseline visit, CVL is performed last. At the follow-up visits, CVL is performed following the collection of the vaginal swabs and prior to the collection of the cervical swabs and Pap smear.

Spray 10 ml of sterile normal saline against the cervical os and the exocervix 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, Catalog # 13-117-9A; pack of 500 @ $23.00). The fluid is then aspirated from the posterior vaginal fornix using the syringe and transferred to a 15 ml sterile polypropylene tube.

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 done. Add volume recovered to 15 ml tube.

CVL fluid samples will be collected at every visit for HPV studies and central and local repositories. HPV testing (PCR and hybrid capture on PCR positives) will be run on CVL specimens collected at Visits 1 through 4. CVL specimens collected after Visit 4 will be saved for future testing. 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.

NOTE: CVL is collected from all women whether or not the cervix is present.

C. CERVICAL SWABS AND PAP SMEAR

1. Pap Smear:

If necessary, gently remove vaginal debris with a large swab. Rotate wooden spatula two complete 360° rotations. Do not spread the spatula on the slide until the brush specimen is also ready. Insert cytobrush gently into cervical os and rotate 360°. Spread the specimen over the slide with the spatula. Immediately follow this procedure by spreading the brush specimen on top of the spatula specimen. Smear the spatula and roll the brush onto a glass slide with frosted end and spray with pump fixative IMMEDIATELY.

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

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

1. Mark the WIHSID and month and year of birth on frosted portion of slide with #2 pencil. Pre-printed labels from a laboratory data management systems may be placed on the outside of the slide mailer but are not required.
2. Complete a *UAB Cytopathology Requisition Form* for each sample at the time of collection; ensure that this requisition is packaged with each slide. The requisition is available in MS Excel format and as many fields should be completed in the electronic form as possible to ensure that information is legible. UAB will print *C60* forms locally; WIHS sites do not need to send *C60* forms in the shipment.

   Record the following information on the requisition:

   - WIHSID and visit number
   - Study site number (1=Bronx, 2=Brooklyn, 3=Washington, DC, 4=Los Angeles, 5=San Francisco, 6=Chicago, 81=Chapel Hill, 82=Atlanta, 83=Miami, 84=Birmingham, 85=Jackson)
   - Collection date
   - Specimen ID from pre-printed laboratory data management system label
   - Participant's age
   - Month, day, and year of the participant's date of birth, for example, mm/dd/yyyy format is 04/01/1974
   - Name and phone number of clinician who collected the slide
   - Physician name should remain "Strickler, H MD"
   - Type of test
   - Source of specimen (cervical, endocervical, vaginal, or other)
   - Complete the "Clinical Information and History" section of the requisition form if known. If you don't know the history, record "unknown" on the form.

NOTE: For visits 1 through 33, WIHS Pap smears were assessed by Dianon. Starting with visit 34, the University of Alabama, Birmingham, Department of Pathology, became the central testing facility.

2. **Swab #6 for 2011/12 and WIHS-V new recruits at baseline (Vaginal) for gonorrhea and Chlamydia NAAT:**

   Local testing facilities have different methodologies and sites should contact the testing facility for instructions. All sites should ensure nucleic acid amplification technology tests are used.

3. **Swab #6 for follow-up visits, #7 for 2011/12 and WIHS-V new recruits at baseline (Cervical) for HPV DNA:**

   The HPV DNA swab should be collected from all participants at every visit beginning with visit 31. This swab should be collected after CVL and Pap smear collection. Supplies are available in the Digene Female Swab Specimen Collection Kit (*50 Dacron swabs and Specimen Transport Medium for cervical specimen collection, Catalogue # 5123-1220*).

   A. AT THE CLINIC:

      1. A dacron swab will be introduced into the os, rotated 180 degrees five times within the endocervix, and then wiped along the external os. Ensure that the portion of the Dacron swab that is left in the tube does not touch surfaces other than the cervix (including hands). If the cervix is missing, then a swab should be similarly obtained (i.e., same number of rotations) from the vaginal pouch in the region of the cervix.
      2. Place the tip of the swab in a tube with 1ml of Digene specimen transport media (included in the kit); break-off the swab at the notched break point.
      3. Swabs should be sent to the lab for processing within 48 hours.

   B. IN THE LAB:

      1. Vortex the tube with the swab in place for 5-10 seconds at a medium setting.
      2. While observing contamination precautions, use a disposable (round) toothpick to begin to remove the swab from the tube (inserting the tip of the toothpick into the hollow end of the swab handle). Lift the swab above the fluid level by the toothpick and let it gently rest against the side of the tube and drain for five seconds before fully removing it. Once the swab is removed use a disposable fine-tip transfer pipette (not standard plugged tips) to extract the fluid from the tube.
      3. Transfer fluid to a 1.8 ml cryovial that is labeled with the appropriate participant and specimen information, approximately 0.6 – 1 ml of fluid.
      4. Freeze sample at -80°C (+/-10°) within 72 hours of sample collection. Ship on dry ice in regular shipments of WIHS core specimens to the Central Repository where samples will be stored for future testing.

   C. ORDERING INFORMATION

      Sites must complete the Qiagen New Customer Credit information form and refer to their assigned Qiagen account numbers when ordering. WIHS sites should obtain a 20% discount.

      - 5123-1220 Individual Female Swab Specimen Collection Kit™
      - 5128-1220 Bulk Medium (STM) CE

Contact: Natasha Ramos
Customer Care Project Leader, QIAGEN, Inc.
19300 Germantown Road
Germantown, MD 20874
Tel: 1-800-426-8157, ext. 23481
FAX: 1-800-718-2056 or 1-240-632-7469
natasha.ramos@qiagen.com
www.qiagen.com

D. ANAL SPECIMEN COLLECTION

Anal specimens will be collected from HHV-8 positive participants at WIHS core visits 10, 11 and 12. Brush one dacron swab over the anorectal area and return to a sterile dry sleeve collector. It is acceptable to use a Dacroswab similar to those used for viral culture and break it off into a sterile, screw cap, plastic 1.5–2 ml tube. Transport the swab at room temperature to the local lab and store at -80°C (+/-10°). It is acceptable to store at -20°C at the local collection site; however, the sample should be archived at -80°C (+/-10°) prior to shipment, if possible. Ship to the Central Repository on dry ice with regular batch shipments of WIHS core specimens. Sites involved in Joel Palefsky's anal HPV substudy will collect one additional Dacron swab from participants enrolled in the substudy.

E. LABORATORY DESIGNATIONS BY TEST

Exam Site	• pH • Trichomonas Culture (Optional) • *T. vaginalis* (Wet Mount) • KOH Prep • Amine (odor) test
Local Immediate	• Gonorrhea and Chlamydia for all 2011/12 and WIHS-V recruits at baseline
Central Immediate	• Pap smear
Local Save & Batch	• Local repository aliquot scheme for CVL is at the discretion of each site once central repository requirements have been satisfied
Central Save & Batch	• BV slide • Syphilis DFA slide at visits 1-30 • CVL • Anal specimens from HHV-8 positive participants only at Visits 10, 11 and 12 or from anal substudy participants • HPV DNA swab for supernatant at visits 31+

V. CENTRAL LABORATORY SHIPMENT SCHEDULE AND DIRECTORY

A. SLIDES FOR BACTERIAL VAGINOSIS

Slides collected through visit 12 have all been shipped to the central lab for testing. All slides collected after visit 12 should be stored at Fisher Bioservices for future testing. Sites sent visit 13-22 slides to Fisher Bioservices during visits 22 and 23. Beginning with visit 23, all BV slides should be sent in a single shipment at the end of each visit. Visit 29 BV slides were sent to the Chicago site (c/o Angela Shansky, 2255 W. Harrison St. Suite C, Chicago, IL 60612) for testing. Visit 30+ slides should be sent to Fisher Bioservices. Sites should email a short shipment notification to Jennifer Weck at jennifer.weck@nih.gov prior to shipment.

Individual slides should be boxed in slide boxes holding 100 slides each. Each box of 100 slides must be labeled with the study name, site name and visits/dates of collection for slides included in the box. Multiple slide boxes may then be placed in a single shipping box, which should be labeled with the same information listed above.

Ship to: ATTN: Jennifer Weck
Fisher BioServices, WIHS
625 Lofstrand Lane
Rockville, MD 20850
Telephone: 301-340-1620
FAX: 301-838-9753

B. PAP SMEARS

Pap smear kits and mailing supplies are purchased by individual WIHS sites. Beginning with visit 34, central Pap smear testing changed from Dianon to UAB. Advance shipment notification and a manifest should be faxed to the UAB laboratory. A manifest should also be included in the shipment. The manifest should include at least the following information:

- Site number (1 – 6, 81 – 85) as follows: 1=Bronx; 2=Brooklyn; 3=Washington, DC; 4=Los Angeles; 5=San Francisco; 6=Chicago; 81=Chapel Hill; 82=Atlanta; 83=Miami; 84=Birmingham; 85=Jackson.
- Shipper's contact person, phone, email, mailing address.
- Total number of Pap smear slides in shipment.
- List eight-digit WIHSIDs and associated two-digit WIHS visit numbers and collection dates for samples in the shipment.
- Local database tracking number on external label, if used.
- Clearly indicate the fax number to send confirmation of shipment receipt.
- Courier name, tracking number, ship date, and expected arrival date.

Shipments should be sent to UAB Monday through Wednesday only, using a courier and delivery option that can track the shipment; sites should contact UAB in advance to make special arrangements for shipment on other days of the week. UAB is open to accept shipments 8:00 am – 5:00 pm, Monday – Friday. Slides should be stored and shipped at room temperature. UAB will confirm receipt of shipment by returning a signed and dated fax. Sites are responsible for tracking shipments to UAB and contacting UAB if a clinical report is not received within two weeks of shipment arrival.

A completed UAB requisition must accompany each Pap smear in the shipment. **It is recommended that the clinician fill out the requisition at the time the Pap smear is obtained. Please be sure to complete all required information on the requisition form, including the "history" section if known, as it affects the pathologic interpretation of the sample.** If you don't know the history, record "unknown" on the form. Contact Allison Wrenn, UAB Cytology Supervisor, allisonwrenn@uabmc.edu.

Shipments should be sent to the following address:

Allison Wrenn CT(ASCP)
UAB Cytology Laboratory
Hospital Support Building - HSB 100
508 20th Street South
Birmingham, Alabama 35294
TELEPHONE: (205) 934-2025
FAX: (205) 975-7056

C. ANAL SUBSTUDY DACRON SWABS

Ship all anal substudy specimens to:

Maria Da Costa
UCSF
Box 0654
513 Parnassus, Room S-420
San Francisco, CA 94143-0654

Contact info to inform Maria Da Costa of impending shipment:
Phone: 415-476-9168
FAX: 415-476-9364
email: maria.dacosta@ucsf.edu (prefers email to fax)

D. CARDIOVASCULAR SUB STUDY CENTRAL TESTING

See instructions in **MOO Section 30** for collection and processing details. Serum, whole blood and plasma will be tested for total cholesterol, HDL-C, LDL-C, triglycerides, glucose, insulin, and HgA1c, depending on visit number and the participant's fasting status at the time of the draw.

If the participant is fasting at even visits, samples should be collected for insulin, triglycerides, lipid panel, glucose, and HgA1c determinations. For non-fasting core blood samples, a modified version of the lipid panel will be used. Site discretion is allowed in performing the non-fasting metabolic panel on a monthly schedule. Sites may elect to keep leftover serum, glucose, and whole blood locally until the central testing lab confirms that testing is completed. Otherwise, leftover serum can be sent to the central repository. Sites will average 1 box per week of specimens for central metabolic testing.

See **Appendix B, Schedule of Laboratory Evaluations,** and **MOO Section 30** for a description of historical testing.

1. SPECIMENS SENT FOR CENTRAL TESTING

   The following samples will be processed and sent to Quest Diagnostics in 1 ml aliquots for testing. The remainder can be stored at the central or local repository.

Tube	Analytes	Collection to centrifuge time	Collection to freeze time	# of Aliquots by fasting status at Core visit	Specimen Code
SST: serum *(10ml tube, 8.5ml draw)*	Metabolic panel‡, TRIG*, insulin*	1 hour	8 hours	F: 1 ml x 3	42
				NF: 1 ml x 2	03
NaF/K oxalate (gray-top) tube: plasma *(3ml tube, 3ml draw)*	Glucose*	30 hours	30 hours	F: 1 ml x 1	43
				NF: N/a	N/a
EDTA (lavender) tube: whole blood *(3ml tube, 3ml draw)*	HgA1c	30 hours	30 hours	F: 1 ml x 1	41
				NF: 1 ml x 1	08

* Will not be tested on non-fasting Core specimens.
‡ Site discretion is allowed in central testing of the non-fasting lipid panel. *Fasting metabolic panel*: total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), calculated low-density lipoprotein cholesterol (LDL-C), triglycerides (TRIG), and insulin. *Non-fasting metabolic panel*: TC, HDL-C, and direct LDL-C.

2. SHIPMENT MANIFEST

   A manifest template in Microsoft Excel was provided to sites at the beginning of visit 20. The following fields must be in the manifest:

   a. Unique vial identifier
   b. WIHSID
   c. WIHS Visit
   d. Date of Collection
   e. WIHS Specimen Code
   f. Volume
   g. Quest Diagnostics Incorporated test number (fasting panel is 81892; non-fasting panel is 81876)
   h. Protocol Number (WIHS)
   i. Box
   j. Row
   k. Column

   The following fields can be provided in the manifest but are not required:

   a. LDMS primary, additive, and derivative codes
   b. Custom, site-specific identifier

3. SHIPMENT NOTIFICATION

   Sites must notify Quest at least 24 hours prior to shipment with a completed fax (see **Appendix C**).

4. SHIPPING REQUIREMENTS

   Sites are responsible for shipping materials and the cost of shipping. All vials for a participant at a visit should be grouped in adjacent locations within a box. It is preferable for a complete set of participant vials to remain in one box. Depending on the fasting status of the participant, 16-27 complete sets should fit in one box. A set of a participant's vials should not be sent in multiple shipments. Boxes within a shipment should have a unique identifier and ideally all boxes sent to Quest should have a unique identifier across all shipments. Prior to shipment, sites should verify that barcodes are readable and that vials match the manifest.

5. SHIPPING SCHEDULE

All specimens collected for metabolic testing should be shipped to Quest once a month. Shipments can arrive between Tuesdays and Fridays only. Sites are expected to adhere to the agreed-upon shipping schedule and notify Quest if they will not ship in their designated week.

Week of the month	Site that will ship to Quest
First	Brooklyn
Second	San Francisco
Third	Chicago
Fourth	Los Angeles/Hawaii

6. SHIPPING ERRORS AND CONFIRMATION OF RECEIPT

Quest will not be able to immediately confirm receipt of shipment if there are no problems with the shipment. Sender contacts listed on the notification fax will be contacted in the event of a problem with the shipment, manifest, or vials. Received shipments will be summarized monthly by Quest.

VI. MASTER SUPPLY LIST

A. CLINICAL

- ***All WIHS supplies are to be purchased locally except the HPV DNA cervical swab kit (Digene).***

Phlebotomy:

- centrifuge (horizontal rotor / swing-out head)
- refrigerator (or cooler) for storage of specimens, if applicable
- gloves
- alcohol prep pads
- gauze squares
- bandages
- tourniquets
- blood collection vacuum tubes as noted on the grid (site specific volume & number needed):
  - 1–9 red-top or serum-separator tubes, depending on visit # and local lab volume required
  - 1 (10 ml) tiger or gold top SST
  - 3-4 Lavender tubes with and without a gel separator
  - 5 (10 ml) CPT
  - 1 (3 ml) gray-top tube
- blood drawing needles & holders
- 25 g butterfly needle w/ tubing (alternative)
- arterial stick supplies (alternative)
- sharps disposal containers
- biohazard red bags
- labels (biohazard, frozen, PID, etc.)

Physical Exam:

- balance scale with height bar

- standard stethoscope
- blood pressure cuffs in four sizes (pediatric, adult, large, thigh)
- cloth or disposable paper measuring tape
- Bioelectric impedance analysis machine – RJL, model #BIA-101Q or Quantum II
- Dinamap BP monitor
- 128 Hz tuning fork and large Queen's Square hammer for Neuropathy exam

Oral Exam (core): discontinued at visit 21

Skin Exam: none – discontinued at visit 23

Breast Exam: none

Lymph Node Exam: none

Gynecology Exam:

- refrigerator (or cooler) for storage of specimens, if applicable
- exam table to place participant comfortably in lithotomy position
- examination gloves
- speculum (appropriately sized for participant, warmed with water ONLY)

Vaginal Specimens:

- 1 dacron tipped swab (swab #1)
    - pH paper (ColorpHast indicator sticks with a range of 4–7)
- optional sterile dacron swab (swab #2) for trichomonas culture
- 1 dacron swab (swab #3) for Bacterial Vaginosis:
    - 1 frosted glass slide
    - 95% alcohol
    - lead pencil
- 1 dacron swab (swab #4) for the following tests:
    - 1 glass slide, two drops NS (T. Vag. Wet Mount)
    - 1 glass slide & cover slip, two drops 10% KOH
- 1 dacron swab (swab #5) for HPV DNA
    - 5123-1220 Digene Individual Female Swab Specimen Collection Kit™
    - 5128-1220 Digene Bulk Medium (STM) CE

Pap smear:

- Pap kits with fixative include – Fisher Scientific part # 14-372-26, manufactured by Andwin Scientific part # 230110, case of 500 kits with 1 slide, cytology brush, plastic scraper, and fixative. A small supply of Dacron swabs should also be available. Catalog price is $442.49 or equivalent.
- Mailmaster® Mailing Boxes. P7 (6 3/4" × 4 5/8" × 2 1/2") 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. Minimum order of $100; 50/$0.83 each, 100/$0.74 each, 500/$0.66 each. Alternatively, sites may use lighter cardboard slide holders and bubble wrap.

CVL Specimen Collection Kit:

- 10 cc syringe
- 10 ml single dose bottle of sterile NaCl (.9NS)

- 15 ml conical tube (sterile polypropylene)
  - 2nd lavage requires use of 5 ml sterile .9 NS & special syringe apparatus
- 2-inch, 18-gauge angiocath type Teflon catheter or plastic transfer pipette (Fisher brand disposable graduated transfer pipette, Catalog # 13-117-9A; pack of 500 @ $23.00)
- water-based lubricant
- examination gloves
- clean towelette for participant

Anal Specimens:

- 1 dacron swab

GYN Microscopic Evaluation (after exam):

- microscope to read KOH Mount for Yeast & Wet (Normal Saline) Mount for *T. Vaginalis*

Urine Tests:

- clean towelette for participant
- 1 sterile container with screw cap
- pregnancy test
- sterile conical transport tube
- gloves for handling specimen
- PBS or HBSS

B. LABORATORY

Prepared for WIHS based on sample processing supplies used at Dr. Kovacs' LAC+USC lab (as of 10/01/94).

ITEM	COMPANY	CAT/CODE#
Falcon Tubes (16x125 mm)(case of 500)	Becton Dickinson	2037
Unifit Disposable Plastic Pipet Tips (200-1000 uL) (1000 per package)	Rainin	RT 200
Unifit Disposable Plastic Pipet Tips (up to 250 uL) (1000 per package)	Rainin	RT 20
Sarstedt 2 mL screw-cap Microtubes (case of 1000)	Sarstedt	72.693.005
50 mL polystyrene Centrifuge Tubes (rack of 25 tubes)	Fisher	05-539-10
Corning Disposable Sterile Cryogenic Vials (2 mL capacity) (case of 500)	Fisher	09-761-71
Sterile Plastic Individually Wrapped Graduated Pipets (case of 4000)	Fisher	13-711-20
Fetal Bovine Serum (500 mL)	Irvine Scientific	3000
Microcentrifuge Tubes (1.5 ml capacity) (case of 2 packs/250 each)	Fisher	05-541-13
Polyester Protective Tape (liquid nitrogen safe) 33m x 25mm (pack of 36)	Fisher	11-867A
Disposable Laboratory Towels (case of 500)	CMS	073-304
RPMI 1640 (500 ml)	Irvine Scientific	9160
Cover Slips 24 x 0 (one ounce)	VWR	
Kimwipes (case of 2940)	Fisher	06-666-1A

ITEM	COMPANY	CAT/CODE#
Disposable Corning Sero Pipets 1 ml (case of 500)	VWR	53284-391
Disposable Corning Sero Pipets 5 ml (case of 400)	VWR	53284-427
Disposable Corning Sero Pipets 10 ml (case of 400)	VWR	53284-449
Disposable Corning Sero Pipets 25 ml (case of 400)	VWR	53284-460
Sleeves Style 2502 (100 pair) (Abanda Cat # 21921-575)	VWR	21921-575
DMSO (5 five-mL bottles)	Sigma	D 2650
Sterile Gauze 2 x 2 Sponges (case of 2400)	Baxter	B3063-2
Latex X-AM Gloves (Non-Sterile) (cases of 1000)		
Small	VWR	32917-875
Medium	VWR	32917-897
Large	VWR	32917-911
Alcohol Swabs		
(each tray)	Baxter	B3062
(case of 2000)	Fisher	02-665-400
(case of 4000)	CMS	246-073
96 Flat-bottom Wells (100 per case)	Costar	3590
GIBCO PBS 7.4	Gibco	10010015
Wood & Cardboard Slide Boxes (Holds 100 slides)	Fisher	03-445
Yeast Extract	Difco	0127-01-7
Peptone	Difco	0118-17-0
Dextrose	Difco	0155-17-4
Glycerol		G-7757
Nonldet P-40 (non-ionic detergent)	Sigma	N6507
Sarstedt Disposable Cups		72.694.006
Filter Apparatus	Fisher	VFA0250012
CPT	Becton-Dickinson	362761

[i] Lawrence, J. Preanalytical Variables in the Coagulation Laboratory, Laboratory Medicine. Jan 2003. N1, vol 23. Pgs 49-69.

[ii] CLSI. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Sixth Edition. Nov 2007. H3-A6, vol. 27, no.26.

[iii] Ernst, DJ and Calam, R. NCCLS simplifies the order of draw: a brief history. Medical Laboratory Observer. May 2004.

[iv] Ernst, DJ and Szamosi, D. Specimen-collection standards complete major revisions. Medical Laboratory Observer. Feb 2005.

[v] Water moves from the vessels to the interstitium and can reduce the plasma volume by 12%. This concentration of cells and other material will could elevate the platelet count. Lawrence, J. Preanalytical Variables in the Coagulation Laboratory, Laboratory Medicine. Jan 2003, n1, vol 23. Pages 49-69.

[vi] Portions adapted from the Johns Hopkins Hospital Oncology Phlebotomy & Procedure Manual. Clinic staff should also refer to the 4th ed. Phlebotomy Handbook by Garza and Becan-McBride.

[vii] Procedures for BD Vacutainer® CPT™ Cell Preparation Tube with Sodium Citrate. http://www.bd.com/vacutainer/products/molecular/citrate/procedure.asp

[viii] Boyum A. Isolation of mononuclear cells and granulocytes from human blood, Scand. J. Clin. Lab. Invest. 21:77-89 (1968).

[ix] Determine the RPM speed for an individual centrifuge by measuring the radius (measure from the axis of the rotor to the bottom of the bucket) in centimeters. Input the radius measurement and G-force into an RPM calculator. http://www.bd.com/vacutainer/products/molecular/citrate/procedure.asp

[x] Ensure that PBS or HBSS has a pH between 7.2 and 7.4. Substantial cell lysis occurs if the pH is below 7.0 resulting in lowered recovery.

[xi] The resuspended cells can be checked on a Coulter counter or a Hemacytometer to obtain cell concentration: (Count/ml) * (Volume of Resuspended Cells) = Total Cells.

[xii] The resuspended cells can be checked on a Coulter counter or a Hemacytometer to obtain cell concentration: (Count/ml) * (Volume of Resuspended Cells) = Total Cells.

[xiii] When adding freezing media, freeze the cells as quickly as possible after resuspension. The recommended cell concentration per ml/vial is 10E7/ml. If there is a leftover of less than 10E7/ml cells the last aliquoted vial should contain no less that 6x10E7 PBMC cells/ (minimum acceptable).

[xiv] "Mr Frosty" Guide: http://www.nalgenelabware.com/products/productDetail.asp?product_id=405. Nalge/NUNC Cryopreservation Manual: http://www.nalgenelabware.com/techdata/technical/cryo.pdf

Appendix A. Standardized WIHS Specimen Codes, Volumes, and LDMS Codes

WIHS				LDMS☼					
SPECIMEN TYPE	ALPHA CODE	S- CODE	EXPECTED TUBE QUANTITIES	Primary	Additive	Derivative	Sub/ Der	Primary Time Unit	Volume§
Serum (red-top or SST)	S	1	500, 1000, 1800	BLD	NON	SER	-	-	0.5∞, 1, 1.8
				BLD	SST	SER	-	-	0.5∞, 1, 1.8
Serum (tiger-top or gold SST, non-fasting)	NTS	3	500, 1000, 1800	BLD	SST	SER	-	-	0.5∞, 1, 1.8
Plasma (CPT)	TP	4	500∞, 1000, 1800	BLD	CPS	PL2	-	-	0.5∞, 1, 1.8
Viable Cells (CPT)	TC	6	1x10E7*, 6x10E6 ♦, 5x10E5 ♦	BLD	CPS	CEL	DMS	-	10.0
Whole blood (lavender, non-fasting)	NWB	8	500	BLD	EDT	BLD	-	-	0.5
Plasma (lavender, fasting)	FEP	9	500, 1000	BLD	EDT	PL1/PL2	-	Fasting	0.5∞, 1
				BLD	PPT	PL1/PL2	-	Fasting	0.5∞, 1
Dry Cell Pellets (CPT)	TCP	10	5x10E5*	BLD	CPS	PEL	-	-	0.5
Urine Supernatant	U	12	1000‡, 1500	URN	NON	FLD	-	-	1, 1.5
Chlamydia LCR swab	CLCR	21	1†	CER	NON	SWB	-	-	1.0
Urine, clean void	ULCR	22	1000, 5000	URN	NON	URN	-	-	1.0, 5.0
Oral Fungal Culture	OF	23	1†	ORL	NON	SWB	FUN	-	1.0
Vaginal Fungal Culture	VF	24	1†	VAG	NON	SWB	FUN	-	1.0
Whole CVL fluid	CVL	25	1000, 1500	CVL	NON	CVL	-	-	1, 1.5
CVL supernatant	CVS	26	500, 1000	CVL	NON	FLD	-	-	0.5, 1.0
CVL pellet	CVP	27	250*	CVL	NON	PEN	NSL	-	0.2
Stimulated Saliva	SS	28	1000	SAL	NON	SAL	-	-	1.0
Oral Fungal Culture (for Oral protocol)	OD	30	1†	ORL	NON	SWB	FUN	-	1.0
Plasma (HHV-8; SF only)	HTP	31	500, 1000	BLD	ACD	PL2	-	-	0.5, 1
Serum (HHV-8; SF only)	HS	32	500, 1000	BLD	NON	SER	-	-	0.5, 1
Stimulated Saliva (HHV-8)	HSS	33	1000	SAL	NON	SAL	-	-	1.0
Anal specimens (HHV-8)	HA	34	1†	REC	NON	SWB	-	-	1.0
ACD tube viable cells (HHV-8; SF only)	HTC	35	600*	BLD	ACD	CEL	DMS	-	6.0
ACD tube cell pellets (HHV-8; SF only)	HTCP	36	50*	BLD	ACD	PEL	-	-	1.0
Saliva cells (HHV-8)	SSC	37	50*	SAL	NON	CLN	DMS	-	1.0
Saliva Supernatant (HHV-8)	SSS	38	500	SAL	NON	FLD	-	-	0.5
Cervical Swab for HIV RNA quantitation	CS	39	1†	CER	NON	SWB	GIT	-	1.0 ±

Appendix A. Standardized WIHS Specimen Codes, Volumes, and LDMS Codes

WIHS				LDMS☼					
SPECIMEN TYPE	ALPHA CODE	S- CODE	EXPECTED TUBE QUANTITIES	Primary	Additive	Derivative	Sub/ Der	Primary Time Unit	Volume§
Whole blood (lavender, fasting)	FWB	41	500	BLD	EDT	BLD	-	Fasting	0.5
Serum (tiger-top or gold SST, fasting)	FTS	42	500, 1000, 1800	BLD	SST	SER	-	Fasting	0.5∞, 1, 1.8
Plasma (gray-top, fasting)	FGP	43	500	BLD	SPO	PL1/PL2	-	Fasting	0.5∞, 1
VRS Plasma (CPT)	VTP	44	500, 1000	BLD	CPS	PL2	-	-	0.5, 1.0
VRS Plasma (EDTA): 0 minutes	V00	45	500, 1000	BLD	EDT	PL1	-	-	0.5, 1.0
VRS Plasma (EDTA): 30 min.	V30	46	500, 1000	BLD	EDT	PL1	-	-	0.5, 1.0
VRS Plasma (EDTA): 60 min.	V60	47	500, 1000	BLD	EDT	PL1	-	-	0.5, 1.0
VRS Plasma (EDTA): 120 min.	V120	48	500, 1000	BLD	EDT	PL1	-	-	0.5, 1.0
VRS Viable Cells (CPT)	VTC	49	600*	BLD	CPS	CEL	DMS	-	0.6
Metabolic Substudy: 00 min plasma	MP0	50	1000	BLD	SPO	PL1	-	-	1.0
Metabolic Substudy: 30 min plasma	MP3	51	1000	BLD	SPO	PL1	-	-	1.0
Metabolic Substudy: 60 min plasma	MP6	52	1000	BLD	SPO	PL1	-	-	1.0
Metabolic Substudy: 90 min plasma	MP9	53	1000	BLD	SPO	PL1	-	-	1.0
Metabolic Substudy: plasma: 120 min	MP12	54	1000	BLD	SPO	PL1	-	-	1.0
Metabolic Substudy serum: 00 min	MS0	55	2000	BLD	SST	SER	-	-	2.0
Metabolic Substudy serum: 30 min	MS3	56	2000	BLD	SST	SER	-	-	2.0
Metabolic Substudy serum: 60 min	MS6	57	2000	BLD	SST	SER	-	-	2.0
Metabolic Substudy serum: 90 min	MS9	58	2000	BLD	SST	SER	-	-	2.0
Metabolic Substudy serum: 120 min	MS12	59	2000	BLD	SST	SER	-	-	2.0
Metabolic Substudy whole blood	MWB	60	2500	BLD	EDT	BLD	-	-	2.5
HTLV 1 & 2 re-directs	HTLV	61	500	-	-	-	-	-	-
HSV re-directs	HSV	62	500	-	-	-	-	-	-
Swab (from FGP)	SPO	63	1000	BLD	SPO	SWB	-	Fasting	1.0
Saliva Rinse (pellet)	LAP	64	500,000	SAL	NON	PEN	-	-	6.0
Saliva Rinse (whole)	LAR	65	1000	SAL	NON	SAL	-	-	1.0
Saliva Rinse (supernatant)	LAS	66	1000	SAL	NON	SAL	-	-	1.0
Saliva	SLA	67	1000	SAL	NON	SAL	-	-	1.0
Saliva, Stimulated (pellet)	SLP	68	500,000	SAL	NON	PEN	-	-	6.0
Saliva, Stimulated (supernatant)	SLS	69	1000	SAL	NON	FLD	-	-	1.0
Saliva, Unstimulated (whole)	ULA	70	1000	SAL	NON	SAL	-	-	1.0
Saliva, Unstimulated (pellet)	ULP	71	500,000	SAL	NON	PEN	-	-	6.0
Saliva, Unstimulated (supernatant)	ULS	72	1000	SAL	NON	FLD	-	-	1.0

Appendix A. Standardized WIHS Specimen Codes, Volumes, and LDMS Codes

WIHS				LDMS☼					
SPECIMEN TYPE	ALPHA CODE	S- CODE	EXPECTED TUBE QUANTITIES	Primary	Additive	Derivative	Sub/ Der	Primary Time Unit	Volume§
Smear, Slide	BV	73	1	VGL	NON	SLD	-	-	1.0
Tissue, Slide	DFA	74	1	TIS	NON	SLD	-	-	1.0
Vaginal Tissue	VGL	75	1	VGL	NON	TIS	-	-	1.0
PAP Smear	PAP	76	1	CER	NON	SLD	-	-	1.0
Cervical Tissue (ACSR)	CVB	77	1	CER	NON	TIS	-	-	1.0
Breast Tissue (ACSR)	BRL	78	1	BRS	NON	BRS	-	-	1.0
Tissue (perirectal)	REC	79	1	REC	NON	TIS	-	-	1.0
Hair	HAR	80	Clipping	HAR	NON	HAR	-	-	1.0
Urine (pellet)	UPE	81	Varied	URN	NON	PEN	-	-	1.0
Intensive PK study: 00 minute plasma	P0M	82	500, 1000, 1200	BLD	EDT	PL1	-	-	0.5, 1.0, 1.2
Intensive PK study: 30 minute plasma	P30	83	500, 1000, 1200	BLD	EDT	PL1	-	-	0.5, 1.0, 1.2
Intensive PK study: 60 minute plasma	P60	84	500, 1000, 1200	BLD	EDT	PL1	-	-	0.5, 1.0, 1.2
Intensive PK study: 2 hour plasma	P2H	85	500, 1000, 1200	BLD	EDT	PL1	-	-	0.5, 1.0, 1.2
Intensive PK study: 2.5 hour plasma	P25H	86	500, 1000, 1200	BLD	EDT	PL1	-	-	0.5, 1.0, 1.2
Intensive PK study: 3 hour plasma	P3H	87	500, 1000, 1200	BLD	EDT	PL1	-	-	0.5, 1.0, 1.2
Intensive PK study: 4 hour plasma	P4H	88	500, 1000, 1200	BLD	EDT	PL1	-	-	0.5, 1.0, 1.2
Intensive PK study: 5 hour plasma	P5H	89	500, 1000, 1200	BLD	EDT	PL1	-	-	0.5, 1.0, 1.2
Intensive PK study: 6 hour plasma	P6H	90	500, 1000, 1200	BLD	EDT	PL1	-	-	0.5, 1.0, 1.2
Intensive PK study: 8 hour plasma	P8H	91	500, 1000, 1200	BLD	EDT	PL1	-	-	0.5, 1.0, 1.2
Intensive PK study: 12 hour plasma	P12	92	500, 1000, 1200	BLD	EDT	PL1	-	-	0.5, 1.0, 1.2
Intensive PK study: 15 hour plasma	P15	93	500, 1000, 1200	BLD	EDT	PL1	-	-	0.5, 1.0, 1.2
Intensive PK study: 18 hour plasma	P18	94	500, 1000, 1200	BLD	EDT	PL1	-	-	0.5, 1.0, 1.2
Intensive PK study: 21 hour plasma	P21	95	500, 1000, 1200	BLD	EDT	PL1	-	-	0.5, 1.0, 1.2
Intensive PK study: 24 hour plasma	P24	96	500, 1000, 1200	BLD	EDT	PL1	-	-	0.5, 1.0, 1.2
Left Ventricular Function study: Plasma (lavender)	LVFP	97	500, 1000	BLD	EDT	PL1	-	-	0.5, 1.0
Herpes Swab	HS	98	1	GLU	NON	SWB	VTM	-	1.0
Anal Cytology	AC	99	1	REC	UNK	CIO	-	-	1.0
Urine Culture	UC	100	1	URN	NON	URN	-	-	1.0
ACSR ACD Plasma	07-Y	101	1000	BLD	ACD	PL1	-	-	1.0
ACSR ACD PBMC	08-Y	102	1000	BLD	ACD	CEL	DMS	-	10.0

Appendix A. Standardized WIHS Specimen Codes, Volumes, and LDMS Codes

WIHS				LDMS☼					
SPECIMEN TYPE	ALPHA CODE	S- CODE	EXPECTED TUBE QUANTITIES	Primary	Additive	Derivative	Sub/ Der	Primary Time Unit	Volume§
ACSR Cervical Control Tissue	CERC	103	1	CER	NON	TIS	TFM	-	1.0
ACSR Cervical Lesion Tissue	CERL	104	1	CER	NON	TIS	TFM	-	1.0
ACSR Vaginal Control Tissue	VAGC	105	1	VGL	NON	TIS	TFM	-	1.0
ACSR Vaginal Lesion Tissue	VAGL	106	1	VGL	NON	TIS	TFM	-	1.0
ACSR Vulva Control Tissue	VULC	107	1	VUL	NON	TIS	TFM	-	1.0
ACSR Vulva Lesion Tissue	VULL	108	1	VUL	NON	TIS	TFM	-	1.0
Oral Rinse (Scope or Saline)	SCR	109	50,000	SAL	OTH	SAL	-	-	50.0
Plasma (EDTA, HIV RNA)	EPH	110	1000	BLD	EDT	PL2	-	-	1.1
				BLD	PPT	PL1	-	-	1.1
Urine Pellet	UPN	111	1000	URN	NON	PEN	PBS	-	1.0 †
HPV DNA swab supernatant	HDS	112	600 - 1000	CER	NON	SWB	DTM	-	0.6-1.0
2011/12 recruits: HCV+ testing	IVHC	113	1000	BLD	NON	SER	-	-	1.0
				BLD	SST	SER	-	-	1.0
2011/12 recruits: Sex steroids	IVSS	114	1000	BLD	NON	SER	-	-	1.0
				BLD	SST	SER	-	-	1.0
2011/12 recruits: hsCRP	IVHS	115	500	BLD	NON	SER	-	-	0.5
				BLD	SST	SER	-	-	0.5
2011/12 recruits: Renal testing	IVRT	116	1000	URN	NON	URN	-	-	1.0
MSK substudy: serum	MSKS	117	1200	BLD	NON	SER	-	-	1.2
				BLD	SST	SER	-	-	1.2
MSK substudy: viable cells	MSKC	118	1x10E7*	BLD	CPS	CEL	DMS	-	10.0
HPV substudy: sodium heparin tube∆	HPH	119	10,000	BLD	HEP	BLD	-	-	10.0
CCSS Substudy Thin-Prep Pap #1	TP1	120	1†	-	-	-	-	-	1.0
CCSS Substudy Thin-Prep Pap #2	TP2	121	1†	-	-	-	-	-	1.0

* Reflects cell concentration in each aliquot and is not a liquid measurement.

† Reflects quantity of vials, not volume.

‡ Collected at visits 1-7, at odd visits 31-35, and at even visits 36+

☼ The "Other Specimen ID" field should be entered with a three digit WIHS S-code, add one or two leading zeroes as necessary.

§ Volume cannot exceed three characters. One character is reserved for the decimal point.

± Cervical Swab for HIV RNA quantitation should have a volume unit of "N/A" in the LDMS. Swab collected at visits 12-28.

♦ The concentration of viable cells was increased from 6x10E6 to 1x10E7 at visit 22. Viable cells with a concentration of 5x10E5 were discontinued at visit 27.

∞ Starting with visit 27, CPT Plasma was placed only in 1ml aliquots. Prior visit protocols have specified 0.5 ml. Starting with visit 29, all core serum and plasma were placed only in 1ml aliquots. Starting with visit 36, one vial each of core plasma and serum was filled to capacity (1.8 ml).

APPENDIX B: WOMEN'S INTERAGENCY HIV STUDY - SCHEDULE OF LABORATORY EVALUATIONS

TEST	FORM #	BASELINE WIHS V Cohort	VISIT 41 Oct 2014 - Mar 2015	VISIT 42 Apr 2015 - Sept 2015
Blood				
HIV ELISA	L01	X HIV- or indeterminate, or HIV+ with no documentation	X HIV- or indeterminate only	X HIV- or indeterminate only
Western Blot	L01	X + ELISA only	X + ELISA only	X + ELISA only
Automated CBC / Differential	L03	X	X HIV+ only	X
Manual Differential	L03A	X if automated diff is flagged	X if automated diff is flagged	X if automated diff is flagged
T-Cell count & subsets	L04	X	X HIV+ only	X HIV+ only
Liver/Renal Function Partial Chemistries	L05	X	X	X
RPR Syphilis Screening	L06	X		
Hepatitis Serology	L02	X		
HIV RNA Quantification	C54	X HIV+ only	X HIV+ only	X HIV+ only
Serum, Plasma & Cells for central & local repository	F10, L20	X	X	X
Lipid panel		X		X
Insulin and glucose		X if fasting		X if fasting
HgA1c		X		X
ACSR ACD cells (only if pelvic biopsy obtained)		X as per protocol	X as per protocol	X as per protocol
Special event additional collection	L20		X as per protocol	X as per protocol
MSK sub study	L20		X as per protocol	X as per protocol

APPENDIX B: WOMEN'S INTERAGENCY HIV STUDY - SCHEDULE OF LABORATORY EVALUATIONS

TEST	FORM #	BASELINE WIHS V Cohort	VISIT 41 Oct 2014 - Mar 2015	VISIT 42 Apr 2015 - Sept 2015
Urine				
Urinalysis	L10	X local immediate		
Urine Pregnancy	F31/F31r	X	X	X
Urine for renal testing for repository	L20	X		
Urine Pellet for repository	L20	X		X
Urine Supernatant for repository	L20	X		X
Vaginal Swabs & Samples				
pH, T.vaginalis (wet mount), KOH, Amine Odor test	F08	X	X	X
Trichomonas Culture	L18	optional	optional	optional
Bacterial Vaginosis	C45	X Save & batch	X Save & batch	X Save & batch
Cervical Swabs				
Chlamydia: Gen-Probe (94/95), LCR (01/02), NAAT (11/12)	L09	X local immediate		
Gonorrhea: Gen-Probe (94/95), LCR (01/02), NAAT (11/12)	L13	X local immediate		
Pap Smear	C60	X	X	X
HPV DNA swab supernatant		X Save & batch	X Save & batch	X Save & batch
Cervico-Vaginal Lavage				
HPV by PCR	C52	X collect & save	X collect & save	X collect & save
HPV by Hybrid Capture	C53	X collect & save	X collect & save	X collect & save
CVL for central & local repository	F10, F31, L20	X	X	X
Other clinical exams				
Colposcopy	L14	X as per protocol	X as per protocol	X as per protocol
Pelvic Exam Biopsy and Histopathology	L15	X as per protocol	X as per protocol	X as per protocol
ACSR Pelvic Exam Biopsy		X as per protocol	X as per protocol	X as per protocol
Hair collection	F31	X batch & ship at end of visit, HIV+ on ART	X batch & ship at end of visit, HIV+ on ART	X batch & ship at end of visit, HIV+ on ART
Oral Protocol specimens				
ACSR Scope or saline oral rinse (only if pelvic biopsy obtained)		X as per protocol	X as per protocol	X as per protocol

WOMEN'S INTERAGENCY HIV STUDY

Notification of Shipment: Real Time Metabolic Testing

I. Sender Details

(Fax this form one day prior to shipment)

Site Name: _____
Contact Person: _____
Phone #: _____
Fax #: _____
Email address: _____
Project: WIHS Metabolic

II. Recipient Details

William A. Meyer III, PhD
Virology Department
Quest Diagnostics
1901 Sulphur Spring Road
Baltimore, MD 21227

Phone #: 410-247-9100
Virology Department (ext 1713)
Fax #: 410-536-1474

III. Shipment Details

(To be completed by sender)

Courier: _____
Airbill #: _____
Date shipped: _____
Number of Boxes: _____
Number of Vials: _____
Amount of Refrigerant: _____ kg

IV. Comments

_____
_____
_____
_____
_____
_____
_____

APPENDIX D: EXPECTED CENTRAL REPOSITORY ALIQUOTS FOR LDMS PRE-LOADS

Fill as many vials as possible to the stated volume or concentration. Overfilling up to 0.2 ml, but not exceeding the limit of the vial, to account for later discrepancies in volume due to long term storage is acceptable. Ensure that the manifest and vial volumes are in agreement. Note that table does not include specimens for local repository storage.

	Recruit Type	Visit	Specimen	Central Requirement	LDMS CODE: PRI/ADD/DER/Sub	S-CODE†	Fasting Status	Pre-Load
1	Follow-up	Core	Serum	3 x 1mL + 1 x 1.8mL	BLD/NON/SER or BLD/SST/SER	1		Fasting CORE
3	Follow-up	Core	Serum	3 x 1mL + 1 x 1.8mL	BLD/SST/SER	42	Fasting	Fasting CORE
4	Follow-up	Core	CPT Plasma	3 x 1mL + 1 x 1.8mL	BLD/CPS/PL2	4		Fasting CORE
5	Follow-up	Core	CPT PBMC	4 @ 1x10E7 cel	BLD/CPS/CEL/DMS	6		Fasting CORE
6	Follow-up	Core	CPT Dry cell pellets	4 @ 5x10E5 cel	BLD/CPS/PEL	10		Fasting CORE
7	Follow-up	Core	Cervical-vaginal lavage	7 x 1 mL or 4 x 1.5mL	CVL/NON/CVL	25		Fasting CORE
8	Follow-up	Core	Cervical swab supernatant	1 x 0.7mL	CER/NON/SWB/DTM	112		Fasting CORE
9	Follow-up	Core	Urine, supernatant‡	1-2 x 1mL + 1 x 1.5mL	URN/NON/FLD	12		Fasting CORE
10	Follow-up	Core	Urine, pellet with PBS§	1 x 1 mL	URN/NON/PEN/PBS	111		Fasting CORE
1	Follow-up	Core	Serum	3 x 1mL + 1 x 1.8mL	BLD/NON/SER or BLD/SST/SER	1		Non-Fasting CORE
2	Follow-up	Core	Serum	3 x 1mL + 1 x 1.8mL	BLD/SST/SER	3	Non-Fasting	Non-Fasting CORE
4	Follow-up	Core	CPT Plasma	3 x 1mL + 1 x 1.8mL	BLD/CPS/PL2	4		Non-Fasting CORE
5	Follow-up	Core	CPT PBMC	4 @ 1x10E7 cel	BLD/CPS/CEL/DMS	6		Non-Fasting CORE
6	Follow-up	Core	CPT Dry cell pellets	4 @ 5x10E5 cel	BLD/CPS/PEL	10		Non-Fasting CORE
7	Follow-up	Core	Cervical-vaginal lavage	7 x 1 mL or 4 x 1.5mL	CVL/NON/CVL	25		Non-Fasting CORE
8	Follow-up	Core	Cervical swab supernatant	1 x 0.7mL	CER/NON/SWB/DTM	112		Non-Fasting CORE
9	Follow-up	Core	Urine, supernatant‡	1-2 x 1mL + 1 x 1.5mL	URN/NON/FLD	12		Non-Fasting CORE
10	Follow-up	Core	Urine, pellet with PBS§	1 x 1 mL	URN/NON/PEN/PBS	111		Non-Fasting CORE
1	New	Baseline	Serum	2-4 x 1mL; 12 x 0.5mL	BLD/NON/SER or BLD/SST/SER	1		Fasting Baseline
3	New	Baseline	Serum	2-4 x 1mL; 12 x 0.5mL	BLD/SST/SER	42	Fasting	Fasting Baseline
4	New	Baseline	Serum	1 x 1mL	BLD/NON/SER or BLD/SST/SER	113		Fasting Baseline
5	New	Baseline	Serum	1 x 1mL	BLD/NON/SER or BLD/SST/SER	114		Fasting Baseline
6	New	Baseline	Serum	1 x 0.5mL	BLD/NON/SER or BLD/SST/SER	115		Fasting Baseline
7	New	Baseline	CPT Plasma	4-5 X 1mL; 2-3 x 0.5mL	BLD/CPS/PL2	4		Fasting Baseline
8	New	Baseline	CPT PBMC	3-4 @ 10E7 cel	BLD/CPS/CEL/DMS	6		Fasting Baseline
9	New	Baseline	CPT Dry cell pellets	4-8 @ 5x10E5 cel	BLD/CPS/PEL	10		Fasting Baseline
10	New	Baseline	Cervical-vaginal lavage	7 x 1mL	CVL/NON/CVL	25		Fasting Baseline
11	New	Baseline	Cervical swab supernatant	1 x 0.7mL	CER/NON/SWB/DTM	112		Fasting Baseline
12	New	Baseline	Urine, Whole	2 x 1mL	URN/NON/URN	116		Fasting Baseline
13	New	Baseline	Urine, supernatant	3-4 x 1mL	URN/NON/FLD	12		Fasting Baseline
14	New	Baseline	Urine, pellet with PBS	1 x 1 mL	URN/NON/PEN/PBS	111		Fasting Baseline
1	New	Baseline	Serum	2-4 x 1mL; 12 x 0.5mL	BLD/NON/SER or BLD/SST/SER	1		Non-Fasting Baseline
2	New	Baseline	Serum	2-4 x 1mL; 12 x 0.5mL	BLD/SST/SER	3	Non-Fasting	Non-Fasting Baseline
4	New	Baseline	Serum	1 x 1mL	BLD/NON/SER or BLD/SST/SER	113		Non-Fasting Baseline
5	New	Baseline	Serum	1 x 1mL	BLD/NON/SER or BLD/SST/SER	114		Non-Fasting Baseline
6	New	Baseline	Serum	1 x 0.5mL	BLD/NON/SER or BLD/SST/SER	115		Non-Fasting Baseline
7	New	Baseline	CPT Plasma	4-5 X 1mL; 2-3 x 0.5mL	BLD/CPS/PL2	4		Non-Fasting Baseline
8	New	Baseline	CPT PBMC	3-4 @ 10E7 cel	BLD/CPS/CEL/DMS	6		Non-Fasting Baseline

APPENDIX D: EXPECTED CENTRAL REPOSITORY ALIQUOTS FOR LDMS PRE-LOADS

	Recruit Type	Visit	Specimen	Central Requirement	LDMS CODE: PRI/ADD/DER/Sub	S-CODE†	Fasting Status	Pre-Load
9	New	Baseline	CPT Dry cell pellets	4-8 @ 5x10E5 cel	BLD/CPS/PEL	10		Non-Fasting Baseline
10	New	Baseline	Cervical-vaginal lavage	7 x 1mL	CVL/NON/CVL	25		Non-Fasting Baseline
11	New	Baseline	Cervical swab supernatant	1 x 0.7mL	CER/NON/SWB/DTM	112		Non-Fasting Baseline
12	New	Baseline	Urine, Whole	2 x 1mL	URN/NON/URN	116		Non-Fasting Baseline
13	New	Baseline	Urine, supernatant	3-4 x 1mL	URN/NON/FLD	12		Non-Fasting Baseline
14	New	Baseline	Urine, pellet with PBS	1 x 1 mL	URN/NON/PEN/PBS	111		Non-Fasting Baseline
1	Subset	MSK	Serum	2-7 x 1.2mL	BLD/NON/SER or BLD/SST/SER	117		MSK
2	Subset	MSK	PBMCs	3 @ 1x10E7 cel	BLD/CPS/CEL/DMS	118		MSK
1	Subset	SE	Serum	2-4 x 1mL	BLD/NON/SER or BLD/SST/SER	1		SE
2	Subset	SE	Serum	2-4 x 1mL	BLD/SST/SER	3		SE
3	Subset	SE	Serum	2-4 x 1mL	BLD/SST/SER	42		SE
4	Subset	SE	PBMCs	2-3 @ 1x10E7 cell	BLD/CPS/CEL/DMS	6		SE

† **Specimen codes depend on fasting status, collection method, and reserved test designation.**

‡ **Even visits only.**

§ **Visits 38, 42, 46 only.**