WOMEN'S INTERAGENCY HIV STUDY

SECTION 21: PROSPECTIVE VIRAL RESISTANCE AND REBOUND STUDY (VRS)

I. BACKGROUND AND SPECIFIC AIMS

The purpose of this research is to address the long-term epidemiology, clinical outcomes and virologic characteristics of antiretroviral treatment failure in the Women's Interagency HIV Study (WIHS) cohort. Highly active antiretroviral therapies (HAART) have substantially altered the clinical and epidemiological outcome of HIV infection in regions where they are available. Failure of this treatment, which is common in the WIHS cohort, may prove to be the major determinant of HIV-related disease progression in women in the next few years.

Materials and data have already been collected in the WIHS for a retrospective analysis that will determine the incidence and prevalence of viral rebound on HAART and the relative frequency of viral rebound with wild type and PI-resistant viral strains, and to determine the HAART therapy outcomes for women with wild type rebound versus women with rebound of PI resistant virus. In addition, this analysis will use existing WIHS data and specimens to identify clinical and individual characteristics that may be associated with viral rebound on therapy.

This protocol describes methods for a prospective study that will use additional specimens and data collection to perform in-depth assessments of WIHS participants who have experienced some level of immunological response to HAART. In particular the prospective study will examine factors that predict continued immunologic response or failure in a relatively small group of WIHS participants, including studies of drug levels (the first such study in the WIHS). The specific aims of this study (based at five WIHS sites) are to determine:

1. Incidence and time course of immunologic failure among women with an initial response to HAART;
2. Immunologic patterns associated with resistant viral rebound compared with wild-type viral rebound;
3. Whether viral isolates from women who have stable CD4 cell counts and viral rebound are different from wild-type viruses in terms of how they enter and replicate in various important immune cells;
4. Predictors of immunologic and virologic failure including past treatment, characteristics of the women's pre-treatment virus, HIV RNA and CD4 cell counts, adherence, levels of antiretroviral medications, acute illnesses, regimen switches, recreational drug use, and social factors.

II. PROSPECTIVE STUDY METHODS

A. OVERVIEW

The VRS is a prospective study of women with stable or increasing CD4 cell counts or recent HAART initiation, employing both clinical and laboratory data.

B. PARTICIPANT ELIGIBILITY AND ENROLLMENT

Eligible women include WIHS participants at five clinical sites (Bronx, Brooklyn, Chicago, San Francisco and Washington DC) who meet eligibility criteria that are applied in a two-stage screening process. The goal of this process is to identify women on HAART, to verify that their CD4 cell counts are not consistently falling, and to identify women who recently reported HAART use and have not had time to develop a discernible CD4 response to this therapy.

STAGE I: WDMAC PRE-SCREENED GROUPS

Women will be identified by WDMAC using data through visit 11 (V11) with the criteria described in Table 1, to generate lists of three groups of women (to be distributed for each site prior to V13). These groups are summarized in Table 1 as:

- Group 1: Women who initiated HAART on or before v9 (Column 1)
- Group 2: Women who initiated HAART at v10 or v11 (Column 2)
- Group 3: Women who have not reported HAART through v11 (Column 3)

Table 1: STAGE I eligibility screening for the VRS Prospective Study

Group 1: Initiate HAART by V9	Group 2: Initiate HAART V10 or V11	Group 3: No HAART by V11
Initiated PI-HAART on or before V9 • Exclude if PI-HAART is not first HAART(i.e., PI-HAART date is not equal to WDMAC 1st HAART date) • Exclude if 2 or more post-HAART visits have been missed • Exclude if no pre-HAART visit within 2 calendar periods (1 year) of HAART initiation • Exclude if CD4 slope is negative (calculate slope using pre-HAART CD4 and all post HAART CD4s) • Exclude if there are two consecutive visits with CD4 drops totaling at least 30% or if there is a CD4 drop of at least 30% between two visits separated by one missed visit, unless the preceding value was 15% greater than other values • Additional exclusions by manual review of CD4 slopes by SF • Exclude women who have to be interviewed in Spanish	Initiate PI-HAART at V10 or V11 • Exclude if PI-HAART is not first HAART (i.e. PI-HAART date is not equal to WDMAC 1st HAART date) • Exclude if no pre-HAART visit within 2 calendar periods (1 year) of HAART initiation • If PI-HAART was initiated at V10 or V11, participant is eligible • Exclude women who have to be interviewed in Spanish	Never initiated HAART on or before V11 • Exclude if participant missed both V10 and V11 • Exclude women who have to be interviewed in Spanish

STAGE II: SCREENING BY SITES AND SAN FRANCISCO

Each of the sites complete the VRS01 form with data from v12 (available in local data bases) for all women who were determined to be eligible via STAGE I screening. VRS01 form is then faxed to San Francisco (415-476-8528, attn: Claudia Ponath). Site and SF responsibilities are summarized in Table 2, below.

Table 2: Stage II Screening Responsibilities

Sites	San Francisco Coordinator
♦ Complete VRS01 for women who meet STAGE I eligibility criteria as defined in Table 1. ♦ Fax VRS01 to SF coordinator.	♦ Review VRS01 for each potential enrollee. ♦ Verify PI HAART use for Group 3 eligibles. ♦ Assess CD4 slope and trends using data reported by sites for v12 using criteria defined below, for Group 1 and 2 eligibles. ♦ Confirm STAGE II eligibility to site.

Eligibility determination (SF coordinator)

1. VRS01 data are entered into database.
2. Determine Stage II eligibility after examination of CD4 trends in v10, v11, v12 given the following scenarios and criteria:
    - No v12 data is available:
        - Group 1⇒ if no v11 data exist, woman is not eligible.
        - Group 2, initiated PI-HAART at v10⇒ if no v11 data exist, woman is not eligible.
        - Group 2, initiated PI-HAART at v11⇒ eligible if v10 data exist.
        - Group 3⇒ woman is not eligible.
        - All groups⇒ manual review to identify any additional exclusions.
    - v12 data are available:
        - Groups 1 and 2⇒ using v10, v11 and v12 CD4 cell counts, if two consecutive drops totaling 30% or more in CD4 exist, or the total slope of CD4 is negative, the woman is <u>not</u> eligible.
        - Groups 1 and 2⇒ with v10, v12 CD4 counts available and v11 missing: if total slope of CD4 cell count is negative, or if there is a 30% drop in CD4 count between v10 and v12, unless the v10 value was 15% higher than the v9 value, woman is no longer eligible.
        - Group 3⇒ women remain eligible if they began PI-HAART at v12 (per VRS01 form).
        - All groups⇒ manual review to identify any additional exclusions.

C. ENROLLMENT PROCEDURES AND SEQUENCE OF ENROLLMENT VISIT

1. Flag charts of all women who are confirmed eligible by SF and complete question A6 on VRS05.
2. When scheduling core visits, if possible, let eligible women know that they are eligible for a substudy and to expect to spend an extra 30 minutes at the visit.
3. At the v13 core visit, all women whose eligibility was confirmed by study coordinator in San Francisco are asked if they would like to participate in the VRS.
4. Study procedures should be discussed with participants in detail, especially having to take their HAART medication at a study visit and having blood drawn four times in the course of two

hours. If a participant will not be able to comply with study procedure requirements, she should not be enrolled.

5. If participant agrees to participate, obtain informed consent and complete VRS02.
6. Administer VRS03, VRS04 and VRS05.
7. If indicated on VRS05, obtain release for medication records and contact participant's physician to complete VRS06.
8. Describe three-month visits: Need to bring medications to the visit, need to hold dose that precedes the visit to take PI medication at the visit (and other ART meds if she takes them at the same time). Participants who take nighttime doses of ART meds should take them as usual, and make note of the time they take their medicine prior to the first three-month visit.

D. GENERAL CONSIDERATIONS

1. SCREENING FOR CHANGES IN HAART REGIMEN

   - Participants need to be screened for changes in their HAART regimen (defined as either adding or dropping medications of their HAART regimen) with VRS05 for two reasons:
     - So it can be determined whether a visit needs to be scheduled for two-hour pharmacokinetic (PK) testing. Two-hour PK testing will be done after any reported change in HAART, unless a participant reports that she is not taking any PIs or NNRTIs.
     - So it can be determined whether a medication release needs to be obtained and the woman's physician contacted to complete VRS06.
   - All participants need to be screened at all visits.
   - At the recruitment core visits, VRS05 is administered after the other VRS forms. No PK visit needs to be set up at this time, but if indicated, the medication release should be obtained and the woman's physician contacted to complete VRS06.
   - At the first three-month VRS visit, all participants will undergo two-hour PK testing. The screening with VRS05 is to be done after the other forms are completed. If indicated, the medication release should be obtained and the woman's physician contacted to complete VRS06.
   - At all following core visits, VRS05 should be administered at the end of the core interview.
   - At all following three-month VRS visits, the screening with VRS05 should take place either when scheduling the visit over the phone, or, if that is not possible, at the beginning of the visit.

2. SCHEDULING TWO-HOUR PK VISITS ACCORDING TO HAART REGIMEN

   - When scheduling two-hour PK visits, collect detailed information as to what medications the participant takes at what times during the day. Participant should be scheduled within two hours of when she usually takes her medication.
   - Participants who take any PIs should be scheduled according to when they take their PIs.

     Example: Participant takes ddI at 8:00 am and Nelfinavir at noon – schedule around noon.
   - Participants who do not take any PIs, but do take at least one NNRTI:

Schedule participant according to when she takes the NNRTI.

- Participants who take an NNRTI only once a day, at night, (i.e., participant takes Efavirenz at 10:00 pm) **and** cannot be seen at that time: collect only a random PK sample.
- Participants who do not take any PIs or NNRTIs: no PK samples need to be collected.
- Any cases not covered here: Please contact the study coordinator, Claudia Ponath, for instructions (phone 415-502-6290, e-mail: cponath@itsa.ucsf.edu).
- Participants who do not bring their medication to a two-hour PK visit, or already took their medication at home, should be rescheduled. If participants are not willing to reschedule, that visit will be missing. If this is the baseline PK visit, the participant is excluded from the study.

3. SCHEDULING APPOINTMENTS

   a. Three-month VRS visits with two-hour PK or three-month VRS visits where screening was not possible:

      All three-month, two-hour PK visits are to be scheduled within a one-month window beginning 2.5 months after the preceding core visit. If, for example, the core visit takes place on October 15, 2000, then the three-month VRS visit should be scheduled between January 1 and January 31, 2001. If the preceding core visit is missed, participants should be scheduled within a one-month window beginning 2.5 months after the target date for that core visit. Ideally participants should be scheduled for a time within two hours of the time when they usually take their medicine, and told to bring their HAART medicines and any food they take with them to the study visit. If participants take different medicines at different times of day, the scheduling should be anchored on the PI medicine first and on the NNRTI second. A reminder call should be placed on the day before the visit to remind the participant to bring her HAART medications to the visit and to hold off taking them until she is told to at the visit. These visits will take about two and one half hours for the PK testing.

   b. Three-month VRS visits without two-hour PK testing:

      These visits as well are to be scheduled within a one-month window beginning 2.5 months after the preceding core visit. Participants should be told to bring their HAART medicines to the visit, but that they will not have to take it there. These visits will take approximately one hour.

   c. Core visits

      Core visits will continue to be scheduled as per WIHS protocol. If the screening indicates that the woman has added a new HAART medicine or discontinued one, then a visit for two-hour PK testing needs to occur. Participants should be scheduled to return for the two-hour PK visit within two weeks of the core visit, for a time within two hours of the time when they usually take their medicine, and told to bring the HAART medicines and any food they take with them to the study visit. If participants take different medicines at different times of day, the scheduling should be anchored on the PI medicine first and on the NNRTI second. These visits will take about two and one half hours for the PK testing.

4. VISIT NUMBERS

   All data and specimens collected during a core visit, including two-hour PK sampling within two weeks of the core visit are to be labeled with that visit number.

★ **All data collected during three-month interval visits will be labeled with the preceding core visit number. In addition, the indicator for "3 mos VRS visit" should be circled on appropriate forms**. **This indicator will be used to distinguish between a core and a three-month VRS visit.**

5. INCOMPLETE VISITS

Incomplete visits should be rescheduled within two weeks of the original appointment and repeated in their entirety. The reason for this is that VRS questionnaires need to be administered at the time the blood specimen is obtained, as most questions are centered around the specific blood sampling point.

E. VISIT PROCEDURES AND SEQUENCE OF VISIT

1. BASELINE PK VISIT

a. Ascertain that the participant brought her medicine with her. If the participant does not have her medicines with her, the visit should be rescheduled for a time ASAP. If it is your best assessment that the participant will not return for another visit, or is unlikely to bring her medicines with her in the future, please contact the SF coordinator, Claudia Ponath via phone at 415-502-6290 or pager at 415-719-2751. If participant reports that she is not taking any antiviral medications at this time, collect one 8ml or two 5ml EDTA (purple top) tubes, and one 8ml CPT tube and go to e.

b. Collect one 8ml EDTA (purple top) tube on ice for a PK measurement. Collect one 8ml (or two 5ml) EDTA (purple top) tubes, and one 8ml CPT tube. Record date and time of this 0 minute blood sampling on VRS29 Specimen Collection Form. Take care to carefully record time measures on this form because time is a crucial piece of information for PK studies.

c. Instruct the participant to take her HAART medicines as she does at home (with food if that is her routine). Record the time the participant takes her medicines on VRS29.

d. Set an electronic timer for the subsequent blood draw. At 120 minutes after the participant takes her medications, collect one 8ml EDTA tube of blood on ice. Record the exact time of this blood draw on VRS29. If you end up obtaining the blood specimen a few minutes early or late, be sure to record the actual time at which you obtained the blood, not the time you were scheduled to.

e. Administer questionnaires (F22MED, F22HX, Drug Form 1, Drug Form 2, VRS03, VRS04, VRS05) between the time when the participant takes her medication and the second blood draw.

f. If indicated on VRS05, obtain a signed release for release of medication records and contact the participant's medical provider in order to complete VRS06.

2. CORE VISITS (FOLLOWING ENROLLMENT VISIT)

After completing the entire WIHS core interview, administer VRS05. If participant has not changed her ART or she is not currently on any PI/NNRTI, two-hour PK sampling is not indicated. In those cases, follow the instructions for a core non-PK visit below. If participant has changed her ART and currently is taking a PI and/or NNRTI, two-hour PK sampling is indicated. Schedule participant for two-hour PK sampling according to guidelines in Section D2 and follow the instructions for a two-hour PK visit below.

Core Non-PK Visit:

Administer VRS03 and VRS04 at the core visit, following VRS05. For participants who have a change in their ART, obtain a release for medication records, contact provider and complete VRS06.

Core Two-hour PK Visit:

a. Ascertain that the participant brought her medicine with her. If the participant does not have her medicines with her, the visit should be rescheduled for a time ASAP. If it is your best assessment that the participant will not return for another visit, or is unlikely to bring her medicines with her in the future, please contact the SF coordinator, Claudia Ponath, via phone at 415-502-6290 or pager at 415-719-2751.

b. Collect **one 8ml EDTA** (purple top) tube on ice. Record date and time of this 0 minute blood sampling on VRS029 specimen collection form. Take care to carefully record time measures on this form because time is a crucial piece of information for PK studies. **No other blood samples need to be collected at time 00 as all other tests will or can be run on blood collected at the core visit.**

c. Instruct the participant to take her HAART medicines as she does at home (with food if that is her routine). Record the time the participant takes her medicines on VRS29.

d. Set an electronic timer for the subsequent blood draw. 120 minutes after the participant takes her medications, collect one 8ml EDTA tube of blood and place it on ice. Record the exact time of each of these blood draws on VRS29. If you end up obtaining the blood specimen a few minutes early or late, be sure to record the actual time at which you obtained the blood, not the time you were scheduled to.

e. Administer questionnaires (VRS03, VRS04) after the participant takes her medication and before the second blood draw.

f. Obtain a signed release for release of medication records and contact the participant's medical provider in order to complete VRS06.

Core Random PK Visit:

a. A core Random PK visit should be done when a core two-hour PK visit is indicated, but cannot be completed due to logistical problems. This will most likely be the case when a participant switches to a PI or NNRTI that is only taken once a day and they take their dose at night (i.e., Efavirenz). Core random PK visits may be combined with core visits.

b. Collect **one 8ml EDTA** (purple top) tube on ice. Record date and time of this 0 minute blood sampling on VRS29 specimen collection form. **No other blood samples need to be collected at time 00 as all other tests will or can be run on blood collected at the core visit.**

c. Administer questionnaires (VRS03, VRS04).

d. Obtain a signed release for release of medication records and contact the participant's medical provider in order to complete VRS06.

Abbreviated Core Visits

If a VRS participant has an abbreviated visit at a core visit, no VRS forms need to be completed. However, sites should still try to complete the three-month visit with the participant. To

determine whether the participant has changed medications, sites should refer to the last visit the participant completed entirely, not the abbreviated visit. That is if participant did an abbreviated visit for v15, and then comes in for v15.1, the comparison should be between v15.1 and v14.1.

3. THREE-MONTH VRS VISITS

Screen participants according to procedures outlined in Section D1. If participant has not changed her ART and is currently taking a PI and/or NNRTI, conduct a three-month random PK visit. If participant reports a change in her ART and is currently taking a PI and/or an NNRTI, conduct a three-month two-hour PK visit. If participant is not currently taking any PI or NNRTI, regardless of whether there has been a change in ART, conduct a three-month non-PK visit.

Three-month random PK Visit:

a. Schedule participant according to guidelines in Section D3b.

b. Administer F22MED, F22HX, Drug Forms 1 and 2, VRS03 and VRS04.

c. Collect one 8ml EDTA (purple top) tube on ice for a random PK measurement. Collect one 8ml (or two 5ml) EDTA (purple top) tubes, and one 8ml CPT tube.

Three-month Non-PK Visit:

a. Schedule participant according to guidelines in Section D3b.

b. Administer F22MED, F22HX, Drug Forms 1 and 2, VRS03 and VRS04.

c. Collect one 8ml (or two 5ml) EDTA (purple top) tubes, and one 8ml CPT tube.

d. If participant has had a change in ART, obtain a release for medication records and complete VRS06.

Three-month Two-hour PK Visit:

a. Schedule participant according to guidelines in Sections D2 and D3a.

b. Ascertain that the participant brought her medicine with her. If the participant does not have her medicines with her, the visit should be rescheduled for a time ASAP. If it is your best assessment that the participant will not return for another visit, or is unlikely to bring her medicines with her in the future, please contact the SF coordinator, Claudia Ponath, via phone at 415-502-6290 or pager at 415-719-2751

c. Collect one 8ml EDTA (purple top) tube on ice for a PK measurement. Collect one 8ml (or two 5ml) EDTA (purple top) tubes, and one 8ml CPT tube. Record date and time of this 0 minute blood sampling on VRS 29 specimen collection form. Take care to carefully record time measures on this form because time is a crucial piece of information for PK studies.

d. Instruct the participant to take her HAART medicines as she does at home (with food if that is her routine). Record the time the participant takes her medicines on VRS29.

e. Set an electronic timer for the subsequent blood draw. At 120 minutes after the participant takes her medications, collect one 8ml EDTA tube of blood on ice. Record the exact time of each of this blood draw on VRS29. If you end up obtaining the blood specimen a few minutes early or late, be sure to record the actual time at which you obtained the blood, not the time you were scheduled to.

f. Administer questionnaires (F22MED, F22HX, Drug Form 1, Drug Form 2, VRS03, VRS04, VRS05) after the participant takes her medications and before the second blood draw.

g. Obtain a signed release for release of medication records and contact the participant's medical provider in order to complete VRS06.

4. SPECIMEN HANDLING

a. All tubes (EDTA and CPT) should be filled to capacity and gently inverted several times immediately after blood is collected, to ensure that the anticoagulant mixes evenly with the blood.

b. PK specimens (one 8ml EDTA tube per time point, and labeled with the time of draw, see Section E6 below) must be kept on ice while waiting for and during transport to the lab.

c. Specimens for genotyping and viral load (one CPT tube) and specimens for CBC and Flow Cytometry (one 8 ml or two 5 ml EDTA tubes) are kept at ambient temperature.

5. SPECIMEN LABELING

The date and time of specimen collections must be written onto the pre-printed labels (provided by San Francisco). **There will be special labels for the PK samples that will include the time point at which the blood is supposed to be drawn. It is crucial that the specimens be labeled immediately after collection and that the correct label is placed on each timed sample.** If you run out of pre-printed labels, please contact Claudia Ponath at (415) 502-6290.

6. SPECIMEN COLLECTION FORMS

VRS29 should be completed for all specimens collected at three-month VRS visits and at core PK visits.

7. SHIPPING TO THE LOCAL LAB

Specimens should be delivered to the lab within six hours of the blood draw, counting from time 0. The PK specimens should be kept on ice (4°C) during transport. All other specimens can be kept at ambient temperature.

F. LABORATORY PROCEDURES

1. LABELING CONSIDERATIONS

a. Specimens collected at VRS three-month visits will be labeled 13.1, 14.1, 15.1 and 16.1 according to the preceding core visit. PK specimens collected in conjunction with a core visit will be labeled according to the core visit. The labels on the blood collection tubes will specify the visit number.

b. There are unique VRS specimen codes, one for VRS CPT plasma, one for VRS CPT cells and four for VRS EDTA plasma, one for each time point of collection. They are listed in Section 9 of the Manual of Operations. All specimens should be labeled with these unique VRS specimen codes.

c. It is imperative that the PK samples are labeled according to the time point at which they are drawn. The blood collection tubes will specify the time point. Be sure to use the correct specimen code for each time point.

2. LAB SUPPLIES REQUIRED FOR EACH VRS VISIT

   a. CBC and Flow Cytometry (CD4 cell counts) same as for WIHS core.

   b. For viral load, genotyping and PK testing (these are the same supplies that are used for WIHS core specimens):

      - Cryotubes, flat bottomed with screw tops
      - Freezer boxes: 5" x 5" boxes, 2 inches in height
      - Labels: labels should be places on each stored tube and on the inventory sheet
      - Polyester protective tape (liquid nitrogen safe) should be placed over the label

3. PROCESSING AND LABELING

   EDTA tubes for PK:

   a. Centrifuge EDTA tubes at 1,200 x g for 10 minutes.

   b. Sub-aliquot 1.2 ml of plasma into 2 ml cryotubes for a total of two aliquots in two different tubes.

   c. Sub-aliquot remaining plasma in 1 ml or 0.5 ml aliquots, depending on the amount, into cryotubes for central repository. (Example: if 2 ml remain, aliquot 2 x 1 ml; if 1.5 ml remain, aliquot 1 x 1ml and 1 x 0.5 ml.)

   d. Freeze plasma at –80°C.

   e. PK sampling is done at two different time points. It is extremely important to know when the blood was drawn. All aliquots must be labeled immediately after processing with the following:

      Participant Study ID
      Date of Specimen
      Specimen Type (be sure to select the correct code for each time point)
      Visit #

      Polyester tape is placed over the labels.

   CPT tubes for Genotyping and Viral Load:

   a. Please refer to the WIHS Laboratory Specimen Collection and Processing Procedures in Section 9 of the Manual of Operations.

   b. Sub-aliquot 1.2 ml of plasma into cryotube for genotyping.

   c. Sub-aliquot 1.2 ml of plasma into cryotube for viral load testing.

   f. Sub-aliquot remaining plasma in 1 ml or 0.5 ml aliquots, depending on the amount, into cryotubes for central repository. (Example: if 2 ml remain, aliquot 2 x 1 ml; if 1.5 ml remain, aliquot 1 x 1ml and 1 x 0.5 ml.)

   d. Freeze plasma at –80°C.

   e. Sub-aliquot and freeze all viable cells according to Manual of Operations, Section 9.II.H. However, these cells should be labeled with the specific VRS specimen code listed in Section 9 VIII.A.

f. All aliquots must be labeled immediately after processing with the following:

Participant Study ID
Date of Specimen
Specimen Type
Visit #

Polyester tape is placed over the labels.

EDTA tubes for CBC and Flow Cytometry:

CBC and Flow Cytometry are to be performed at the respective local labs following WIHS guidelines outlined in Manual of Operations, Section 9.

4. SPECIMEN PROCESSING FORMS

Form VRS10 should be completed by the lab for all PK specimens.

5. STORAGE

a. Plasma for PK testing, genotyping and viral load is to be stored at the local lab until shipment to central labs. Specimens must be kept frozen at –80°C.

b. Plasma for the central repository is to be stored at the local lab until are shipped to BBI. Specimens must be kept frozen at –80°C.

c. Cells for the central repository are to be stored in liquid nitrogen at the local lab until they are shipped to the central repository at BBI.

6. SHIPPING

a. Schedules:

VRS viral load and PK specimens should be shipped every three months, beginning April 2001, on dry ice. VRS specimens for genotyping should be shipped upon request by the SF study coordinator (we need to have the viral load results first to determine which specimens can be genotyped).

Cells and plasma for the central repository may be added to core WIHS shipments whenever possible, and documented in the usual fashion.

b. Shipping Manifests:

A copy of all invoices going to the central repository should be sent to WDMAC per WIHS guidelines. The structure of electronic files sent to the central repository does not allow for a visit number with a format like 13.1. Therefore, in the electronic file that corresponds to the shipment, “13” should be used for VISIT and ”1” should be used for INTERIM.

Each shipment to a testing lab should be accompanied by an invoice indicating its contents (specimen list), date of shipment, conditions and destination. The specimen list should be prepared in the same format used for shipments to BBI, and printed out in a column format (e.g., Excel). Columns 4 through 9 (FREEZER and RACK) and 14 through 23 (FREEZER ID) may be left blank. An electronic copy of the invoice (specimen list) should be e-mailed to Claudia Ponath (e-mail cponath@itsa.ucsf.edu) at the time of each shipment for tracking. Additionally, a cover letter should be sent with the specimens stating that these are VRS specimens, the total number of specimens and a contact person with phone and pager number.

c. PK samples:

Amount to be sent: 1 x 1.2ml aliquots of plasma per time point per visit per person

Address: Benet Lab
533 Parnassus Avenue U-66
San Francisco, CA 94143

Notation: WIHS VRS specimens

Contact: 415-476-5890
Mike Goldenberg

d. Genotyping samples:

Amount to be sent: 1 x 1.2 ml aliquot of plasma per 3 month VRS visit per person

Address: SFGH Core Virology Laboratory
Jackie Javier
1001 Potrero Avenue
BLDG 100, Room 104
San Francisco, CA 94110

Notation: WIHS VRS specimens

Contact: 415-502-4775 phone
415-206-6016 fax
Jackie Javier

e. VRS viral load testing:

Amount to be sent: 1 x 1.2 ml aliquot of plasma per 3 month VRS visit per person

Address: Specimen Receiving
Larry Penning, VRDL
California State Department of Health Services
850 Marina Bay Park Way
Richmond, CA 94804

Notation: WIHS VRS specimens

Contact: 510-307-8927 phone
510-307-8605 fax
Larry Penning

f. Plasma for Central Repository:

Amount to be sent: remaining aliquots of plasma per visit per person

Address and Notation: See WIHS guidelines for shipping to the central repository. VRS specimens should be shipped with other WIHS specimens whenever possible.

g. Cells for Central Repository:

Amount to be sent: all aliquots obtained from CPT tube

Address and Notation: See WIHS guidelines for shipping to the central repository. VRS specimens should be shipped with other WIHS specimens whenever possible.

G. INTERVIEW FORMS

Below is a list of all formed used in VRS and at which visits they should be completed.

Baseline Core Visit	Baseline PK visit	Core Non PK visits	Core 2 hour PK visits	3 month random PK visits	3 Month non PK visits	3 Month 2 hour PK visits
Questionnaires						
Core Forms	VRSNOTI	Core Forms	VRS03	VRS05	VRS05	VRS05
VRS02	F22MED, F22HX*	VRS05	VRS04	F22MED, F22HX*	F22MED, F22HX*	F22MED, F22HX*
Consent	Drug Forms 1, 2	VRS03	Medication Release	Drug Forms 1,2	Drug Forms 1,2	Drug Forms 1,2
VRS03	VRS03	VRS04	VRS06	VRS03	VRS03	VRS03
VRS04	VRS04		VRS29	VRS04	VRS04	VRS04
VRS05	VRS05			VRS29	VRS06 (if needed)	VRS06
VRS06 (if needed)	VRS06 (if needed)				Medication Release (If needed)	Medication Release
Medication Release (If needed)	Medication Release (If needed)				VRS29	VRS29
	VRS29					
Lab Reports						
Genotyping	Genotyping	Genotyping		Genotyping	Genotyping	Genotyping
L03 or L03a	L03 or L03a	L03 or L03a		L03 or L03a	L03 or L03a	L03 or L03a
L04	L04	L04		L04	L04	L04
Viral load	Viral load	Viral load		Viral load	Viral load	Viral load
	0 min PK level		0 min PK level	Random PK level		0 min PK level
	120 min PK level		120 min PK level			120 min PK level
	VRS10		VRS10			VRS10

* Events reported in F22HX during three-month VRS visits will not be ascertained.

G. 2. INTERVIEW FORMS VISIT 19 AND ON.
Below is a list of all formed used in VRS and at which visits they should be completed.

Core Random PK visits (New recruits only)	Core WIHS Visit (Enrolled participants)	3-month random PK visits (participant reports PI/NNRTI use)	3-month non-PK visits (participant does not report PI/NNRTI use)
Questionnaires			
Core Forms	Core Forms	F22MED, F22HX*	F22MED, F22HX*
VRSNOTI		Drug Forms 1,2,3	Drug Forms 1,2,3
VRS03	VRS03	VRS03	VRS03
VRS04	VRS04	VRS04	VRS04
VRS05	VRS05	VRS05	VRS05
Medication Release		Medication Release (If change in HAART)	Medication Release (If change in HAART
VRS06R		VRS06 (if change in HAART)	VRS06 (if change in HAART
VRS29		VRS29	VRS29
Lab Reports			
L03 or L03a	L03 or L03a	L03 or L03a	L03 or L03a
L04	L04	L04	L04
Viral load	Viral load	Viral load	Viral load
Random PK level	N/A	Random PK level	N/A
		Genotyping (If VL >1,000)	Genotyping (If VL >1,000)

* Events reported in F22HX during 3 month VRS visits will not be ascertained.

H. VRS SUPPLEMENT PROTOCOL – 12/10/01

1. ELIGIBILITY

   All women enrolled as HAART naïve (Question A5 on Eligibility Form = 2) become eligible for the VRS supplement when they start HAART as defined on VRS01r:

either	$\geq$ 2 NRTI	+	($\geq$ 1 PI	and/or	$\geq$ 1 NNRTI)
or	1 NRTI	+	$\geq$ 1 PI	+	$\geq$ 1 NNRTI

2. RECRUITMENT

   There are several options to identify women who start HAART. Sites are encouraged to use as many as necessary, and as often as necessary.

   a. Tell participants about the VRS at their baseline visit and give them a flyer/info sheet. Offer incentive for them to call you when they start HAART.

   b. In between core visits:

      - Send a second flyer in between core visits.
      - Call participants to check whether they've started HAART (by administering VRS01r).
      - For both, offer participant incentive to call you when they start HAART.
      - Sites who are able to get this information from participants' providers should call participants to invite them into the VRS if provider reports that participant is starting HAART.

   c. At each core visit, participants' medication use should be checked via VRS01r and, if participant qualifies, invite her to join the VRS.

      To cut down on unnecessary work, sites are also encouraged to check CD4 counts of the HAART-naïve enrollees and then proceed as follows:

      For women with CD4 counts of > 400, only check participant's medication use at core visits.

      For women with CD4 counts of $\leq$ 400, contact them in between core visits.

3. SCHEDULING AND CONDUCTING THE VRS VISIT

   a. Participants who report starting HAART between core visits:

      Conduct a three-month two-hour PK visit, according to Section II.E.3 of the VRS protocol.

      The earliest this visit can be scheduled is two weeks after participant starts taking HAART. The window for this visit is from two weeks after the preceding core visit to the start of the window of her next core visit.

   b. Participants who first report HAART at a core visit or are already in their core visit window when they report HAART:

      Conduct a core two-hour PK visit (outlined in Section II.E.2 of the VRS protocol) within two weeks of the core visit. If that is not possible, a three-month two-hour PK visit should be scheduled three months after the core visit.

   c. Consent for the VRS should be obtained at the first VRS visit. Also, regardless of when the first VRS visit takes place, the following forms need to be completed:

      - VRSNOTI
      - Medication records release

- VRS06

d. After the first two-hour PK visit (three-month or core), participants will be seen every three months, according to VRS protocol. If their first VRS visit is a three-month two-hour PK visit, they will be seen again at their next regular core visit. If their first VRS visit is a core two-hour PK visit, they will next be seen for a three-month VRS visit.

I. WIHS VISIT 19

1. ENROLLED PARTICIPANTS

As of WIHS visit 19, two-hour PK sampling is being discontinued. Hair samples should be collected on all VRS participants at their three-month visit.

Core isits:

Conduct Core Non-PK visits for all enrolled VRS participants; that is, complete VRS03, VRS04 and VRS05 at the time of the core visit. Complete VRS06 for participants who have had a change in HAART regimen according to VRS05.

Three-month visits:

For VRS participants who report current use of a PI or an NNRTI, conduct a three-month random PK visit: Administer F22MED, F22HX, Drug Forms 1, 2 and 3, VRS03, VRS04 and VRS05.

Collect one 8ml (or two 5ml) EDTA (purple-top) tubes for CBC and flow cytometry. Collect one 8ml EDTA (purple-top) tube on ice for a random PK measurement, and one 8ml CPT tube for viral load and genotyping. Collect hair sample per WIHS protocol.

For participants not currently taking any PI or NNRTI, conduct a three-month non-PK visit (same as the random PK three-month visit, only no PK sample is collected). Collect hair sample per WIHS protocol.

Complete VRS06 for all participants who have had a change in HAART regimen according to VRS05.

2. ENROLLMENT OF NEW PARTICIPANTS

For eligibility and recruitment, see part “H” of protocol.

For participants who first report HAART at a core visit, conduct a Core Random PK visit (collect one 8ml purple-top on ice; complete VRS03, VRS04, VRS05, VRSNOTI and VRS06R).

Ideally, the Core random PK visit will take place on the day of the Core Visit. If that is not possible, it may be completed within two weeks of the core visit.

For a Core Random PK visit, collect one 8ml EDTA (purple-top) tube on ice. Record date and time of this blood sampling on VRS29 specimen collection form. No other blood samples need to be collected as all other tests will or can be run on blood collected at the core visit.

Administer questionnaires (VRS03, VRS04).

Obtain a signed release for release of medication records and contact the participant’s medical provider in order to complete VRS06R.

For participants who first report HAART in between core visits, conduct a three-month random PK visit and complete VRSNOTI and VRS06R.