WOMEN'S INTERAGENCY HIV STUDY
SECTION 24: HAIR COLLECTION PROTOCOL

A. STUDY PURPOSE

The goal of the WIHS Hair Collection Protocol is to investigate adherence to antiretroviral medications by measuring drug levels in hair samples.

B. HYPOTHESES

Ha. Given the importance of therapeutic drug monitoring in HIV infection, the development of a simple, acceptable and accurate method of measuring participant drug levels will represent a major advance in the treatment of HIV.

Hb. Monitoring drug levels in hair will represent a useful measure of long-term compliance to antiretroviral regimens. It will also show efficacy with or even improvement over current adherence monitoring systems.

Hc. Concentrations of antiretroviral drug levels in hair will correlate with virologic responses in the monitoring of HIV infection, including plasma viral loads and the development of viral resistance.

Hd. Concentrations of antiretroviral drug levels in hair will correlate with side effect profiles, with greater problems with side effects occurring in women with 'supratherapeutic' drug concentrations in hair.

He. Use of single hair analysis for drug concentrations will enhance acceptability and facilitate adherence monitoring in the treated HIV population.

C. SCIENTIFIC AIMS

1. To perform a substudy in the WIHS that involves the collection and storage of hair samples during core visits, with the aim of measuring antiretroviral concentrations in hair.
2. To measure nucleoside reverse transcriptase inhibitor, protease inhibitor and nonnucleoside reverse transcriptase inhibitor concentrations in hair samples of women on these medications in a single hair sample. Melanin content of the hair sample will also be measured and the drug levels will be adjusted for the amount of melanin present, as medications bind to eumelanin in the hair.
3. To measure HIV RNA levels in the subpopulation studied and attempt to correlate hair antiretroviral levels with the plasma viral load.
4. To attempt to find a correlation between antiretroviral levels in hair and intensity of side effects from the medications.
5. To perform viral resistance testing on a subset of the women for which hair drug analysis is undertaken; a correlation between antiretroviral drug levels in hair and number of protease and reverse transcriptase gene resistance mutations will be investigated.

D. BACKGROUND

Highly-active antiretroviral (ARV) combinations have favorably altered the morbidity and mortality of HIV infection in the U.S. Although therapeutic drug monitoring of antiretrovirals is not yet routine, suboptimal drug levels have been shown to be major predictors of treatment failure and the development of viral resistance. Strict adherence to HIV medication regimens is of utmost importance in maintaining adequate serum drug concentrations and achieving viral suppression. Adherence to the

often-complex drug combinations can be limited by side effects, substance abuse, unstable living situations, cost considerations, depression, or fears regarding long-term toxicity. Therefore, the assessment of long-term compliance with HIV medication regimens is crucial in monitoring the response to therapy.

Several methods have been evaluated for measuring adherence to ARV, including self-report, pill counting, tracking cap-opening events and measurement of blood and urine drug concentrations. Each method has limitations such as problems of recall bias and memory failure with self-report. The use of medication organizers interferes with pill counts and cap-opening event detectors. Plasma drug levels are sensitive indicators of treatment adequacy; however, blood concentrations reflect medication doses administered only within one to two days of sampling. This method has limited predictive value for long-term treatment outcome. These limitations of current methods for assessing adherence to ARV have led to proposals for more objective, long-term measures of medication compliance.

The utility of measuring drug levels in hair has largely been touted in the forensics literature as a method to assess exposure to drugs of abuse. However, an increasingly recognized potential of hair analysis is in therapeutic drug monitoring. As the concentration of drugs in hair reflects uptake from the systemic circulation over an extended time window (weeks to months), hair analysis provides an advantage over plasma monitoring in assessing long-term compliance with medications. Indeed, preliminary studies have evaluated levels of an HIV protease inhibitor (Indinavir) in participant hair samples and correlated these levels with virologic outcomes, including degree of viral suppression and the accumulation of resistance mutations.

When considering implementation of hair analysis for therapeutic monitoring, the following factors can influence drug levels in hair:

- Age,
- Ethnicity,
- Hair pigmentation and texture,
- Cosmetic treatment of hair,
- Differences in hair growth rate,
- Anatomic site of hair,
- Diffusion of drug along the hair shaft,
- Physicochemical characteristics of the drug and,
- Influences from drug combinations.

As drug concentrations in hair are linearly related to the amount of melanin present in the sample, normalizing drug levels in hair according to the melanin concentration will increase the accuracy of this technique. The testing protocol will control for melanin during our analysis of hair samples and participants will not be asked at this time about their cosmetic treatment of hair. In fact, most of the variability in measuring drug levels in hair in various participants can be controlled through collection of the hair sample from the same spot on the scalp, measuring drug concentrations in the segment of hair closest to the scalp, and adjusting the drug level for melanin content.

Although initial studies on hair drug analysis utilized approximately 50-300mg of hair and involved lengthy sample preparation procedures, techniques have recently been developed for measuring drug concentrations in single hair samples. Methods now exist to conduct hair drug analysis in less than ten minutes with minimal sample preparation on a single hair. Monitoring antiretroviral levels in hair may thus become a convenient and important adjunct to the field of HIV therapeutics and further study is merited.

E. PARTICIPANT ELIBILITY AND ENROLLMENT

All participants in WIHS who are HIV positive will qualify for the hair collection protocol. Hair collection should be performed at all core WIHS visits on each HIV-positive participant.

NOTE: Through visit 33, hair samples were collected only from HIV-positive women who had taken antiretroviral medication(s) within four weeks prior to their core visit. Beginning with visit 34, specimens are to be collected from all HIV-positive women, regardless of antiretroviral medication use.

F. SUPPLIES NEEDED FOR HAIR COLLECTION

A few basic supplies will be needed to collect and correctly store the hair sample: hair clips, scissors, aluminum foil, dessicant bags, and ziplock bags.

- Aluminum foil can be ordered from **Quill Diagnostics**. The product is called **Handy Foil Standard Aluminum Foil**, catalogue number 035-11205: 12 inches x 100 feet, $39.99. Alternatively, the foil can be purchased locally, if sites can find a better price. Aluminum foil should be cut into squares approximately 5cm x 5cm and folded into quarters.
- Dessicant bags should be ordered from **U-Line**. Phone: 1-800-295-5510; fax: 1-800-295-5571. The product is called **1/2 g Silica Gel Desiccants**, catalogue number S-8032: 1 pail (6000 bags/container), $133.00.
- Scissors can be purchased locally.
- Hair clips can be purchased locally.
- Ziplock bags should be small and can be purchased locally.

G. HAIR COLLECTION PROCEDURE

Clinicians at each site will collect the hair sample during a core visit on each HIV-positive participant. If the participant is HIV-negative, "Not Applicable" should be noted on the *Specimen Collection Form (F31).* It is recommended that hair samples be collected during the physical exam.

1. Clean the blades of a pair of scissors with an alcohol pad and allow blades to completely dry prior to use.
2. Lift up the top layer of hair from the **occipital** region of the scalp. A hair clip can be used to keep this top layer of hair out of the way. Isolate a small thatch of hair (at least 20 fibers of hair) from *underneath* this top layer of hair from the **occipital** region.
3. Place a small label with the participant's WIHSID over the **distal** end of the hair thatch. The distal end is the portion furthest from the scalp. It is very important to place the label at the distal end as this will distinguish the scalp end from the distal end.
4. Cut the small hair sample off the participant's ahead *as close to the scalp as possible*.
5. The pair of scissors used to collect the hair samples should be cleaned prior to using on each participant. Reclean the blades of the scissors with an alcohol pad and allow blades to completely dry prior to reuse.
6. Unfold the square of aluminum foil and place the cut thatch of hair inside the piece of foil. Refold the foil over to completely enclose the thatch of hair.
7. Place a WIHSID label on outside of the folded piece of foil.
8. Place the folded piece of foil inside a Ziplock bag with a dessicant bag in it and seal the Ziplock bag.

9. Hair samples should be kept at room temperature and in a dark place at each site prior to shipment.

H. HAIR SAMPLE STORAGE AND SHIPMENT

Sites should store all hair samples locally for later shipment at the end of each visit window. Thus, all visit 17 hair samples will be shipped at the end of the visit 17 window (March 31, 2003), and all visit 18 samples will be shipped at the end of the visit 18 window (September 30, 2003). Hair samples can be stored at room temperature and are not biohazardous.

- Hair specimens are to be boxed in order of WIHSID and visit.

- Each packet of hair is to be folded to approximately 3” x 3” and labeled with the WIHSID and visit on the upper portion of the packet.

Indestructo Mailers 50/bundle

Vendor	Phone	Cat. #	price
PackagingPrice.com	888-236-1729	MLR84	29.50
The Box Depot 8473 N. Lilley Road Canton, Michigan 48187	734-453-6986	KRB4	25.04

- An electronic excel file in the following format must be e-mailed to Eileen Wong at eileen.wong@ucsf.edu on the day of shipment along with the shipment tracking number:

CENTER	PID	VNUM	DATE
201	20100321	18	8/12/03

- The specimen bank is to be notified of each shipment by calling 415-476-1481. Please let them know what types of specimens you are sending, what study, and site. Also provide a contact name and number if any problems arise in regard to the shipment and contents.

- Specimens should be shipped to:

AIDS Specimen Bank
521 Parnassus Avenue C-540
San Francisco, CA 94143

Contact: Eileen Wong

eileen.wong@ucsf.edu

415-476-1481