

# **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. WHAT WE KNOW FROM WIHS IV**

- a. Monitoring drug levels in hair in the WIHS represents a useful measure of long-term compliance to antiretroviral regimens. Hair levels of antiretrovirals are more predictive of virologic outcomes in the cohort than self-reported adherence.
- b. Concentrations of antiretrovirals in hair correlate with virologic responses in a longitudinal fashion in the WIHS and could serve as an objective measure of adherence and exposure in other cohort and clinical settings.

### **C. 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. 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.

Hc. The addition of tenofovir (TFV) concentration monitoring in small hair samples will provide an integrated measure of TFV exposure in the cohort, which will be useful for evaluating in relationship to long-term toxicities (e.g. renal and bone outcomes)

### **D. SCIENTIFIC AIMS**

1. To measure protease inhibitor, nonnucleoside reverse transcriptase inhibitor, and integrase inhibitor concentrations in hair samples of women on these medications in a small hair sample.
2. To measure tenofovir concentrations in hair samples of women on TFV-based regimens in order to provide a long-term measure of overall tenofovir exposure for each participant.
3. To correlate hair antiretroviral levels with virologic responses in the cohort in a longitudinal fashion.
4. To attempt to find a correlation between antiretroviral levels in hair and intensity of short-term side effects from the medications.
5. To examine the association of hair antiretroviral concentrations (including the newly integrated TFV levels) integrated over time (areas-under-the-hair-concentration-curves) with long-term HIV treatment outcomes (e.g., cardiovascular, metabolic, neurocognitive, hepatic fibrosis, renal toxicity, bone density loss, etc.).

### **E. BACKGROUND**

Highly-active antiretroviral (ARV) combinations have drastically reduced the morbidity and mortality of HIV infection in the U.S. Although therapeutic drug monitoring of antiretrovirals (ARVs) is not yet routine, suboptimal drug levels have been shown to be major predictors of treatment failure and

the development of viral resistance. Near-perfect 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 use, unstable living situations, cost considerations, depression or other mental illness, or fears regarding long-term toxicity. Therefore, the assessment of long-term compliance with HIV medication regimens is crucial in monitoring response to therapy.

Several methods have been evaluated for measuring adherence to ARVs, including self-report, pill counting, tracking cap-opening events and measurement of blood and urine drug concentrations. Each method has limitations which have limited their use in routine clinical settings. Self-report is frequently used in clinical and research settings to track adherence, but is subject to problems with inaccuracy, a desire to please the provider, recall bias and memory failure. The use of medication organizers interferes with pill counts and cap-opening event detectors. Plasma drug levels reflect medication doses administered only one to two days prior to sampling and have limited predictive value for long-term treatment outcomes. The value of single plasma ARV levels is further limited by the so-called “white coat effect,” in which adherence transiently improves prior to clinic appointments[1], and an inability to define meaningful therapeutic ARV ranges given significant inter-individual PK variability[2, 3]. The substantial limitations of current methods for assessing adherence to ARVs 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. Hair ARV concentrations average daily exposure variability in a manner analogous to glycosylated hemoglobin A1C (HbA1c) providing information on mean daily glucose levels in diabetic monitoring.

During the WIHS III and WIHS IV project periods, with the aid of an independent NIAID-funded RO1 (P.I. Ruth Greenblatt), the San Francisco WIHS group developed assays for measuring commonly-used non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitor (PI) levels in small hair samples. We initiated the collection of small samples of hair (30-40 strands) from HIV-infected participants in the WIHS on antiretroviral therapy during visit 18 and have performed a number of analyses and published a number of manuscripts showing the predictive power of hair concentrations of ARVs on treatment outcomes in the cohort. These analyses include analyzing the longitudinal relationship between atazanavir concentrations in hair and virologic outcomes on HIV therapy[4] (Gandhi M, et al. CID 2011); the association between lopinavir/ritonavir and atazanavir levels in hair and initial virologic response on participants starting protease inhibitor-based therapy[5] (Gandhi M, et al. AIDS 2009); the relationship between nevirapine concentrations in hair and clinical outcomes, including virologic success and toxicities (Gandhi M; manuscript in preparation); the correlation between efavirenz concentrations in hair and pharmacokinetic parameters calculated from WIHS intensive pharmacokinetics data(Gandhi M, et al. JID 2012) [6]; and several methods papers[7, 8]. We have shown that hair concentrations of ARVs are the strongest independent predictor of virologic success in the WIHS[4], as well as other cohort settings[9]. We have also examined the role of hair concentrations of tenofovir in the pre-exposure prophylaxis setting[10] and used hair concentrations of ARVs in infants to predict maternal transfer of ARVs during pregnancy and breastfeeding[11]. Finally, we have now developed methods to analyze concentrations of new ARVs in prevalent use in the cohort in hair (e.g. raltegravir and darunavir) and are working with the Division of AIDS (DAIDS)-quality control program called the Clinical Pharmacology Quality Assurance (CPQA) program to have our hair assay validation reports and standard operating procedures peer-reviewed for expanded use in NIAID-funded clinical trial and observational studies.

The inclusion of hair concentrations of ARVs in WIHS as an objective biomarker of adherence and exposure, given the work performed during WIHS IV, reviewed favorably in the WIHS V NIH review and was elected to continue into the next project period by the WIHS EC. The San Francisco WIHS group had proposed an additional aim to the hair studies during WIHS V, which was to assess tenofovir (TFV) concentrations in hair samples from women on TFV-based HAART. The analysis of TFV levels in hair requires 100 strands of hair, rather than the 30-40 strands required for NNRTI and PI monitoring, so the hair collection protocol in WIHS will require alteration for visit 39 to require the collection of 100 strands of hair for women on TFV-based regimens only. This will be done once per year only, at even-numbered visits.

## F. PARTICIPANT ELIBILITY AND ENROLLMENT

All participants in WIHS who are HIV positive and have taken antiretroviral medications since their most recent study visit will qualify for the hair collection protocol. Hair collection should be performed at all core WIHS visits on each HIV-positive participant reporting use of antiretroviral medications since the last study visit.

**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 were collected from all HIV-positive women, regardless of antiretroviral medication use. From visit 39 onwards, hair samples should only be taken from HIV-positive women reporting use of antiretroviral medication(s) since the last study visit. Women reporting use of a tenofovir-based regimen (drug codes 234, 253, 262, 280, 287) should be asked annually whether 100 strands of hair can be collected, rather than 30-40 strands.

## G. SUPPLIES NEEDED FOR HAIR COLLECTION

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

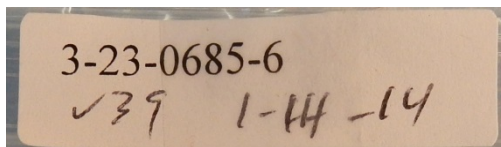
- Aluminum foil can be ordered from **Quill Diagnostics**. The product is called **Handy Foil Standard Aluminum Foil**, catalogue number 035-11205: 12 inches x 100 feet, \$39.99. Alternatively, the foil can be purchased locally, if sites can find a better price. Aluminum foil should be cut into squares approximately 5cm x 5cm and folded into quarters.
- Labels can be purchased locally.
- Desiccant 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. <http://www.uline.com/Product/Detail/S-8032/Desiccants/Silica-Gel-Desiccants-Gram-Size-1-2-5-gal-pail>
- Scissors can be purchased locally.
- Hair clips can be purchased locally.
- Ziplock bags should be ordered from C-Line: C-Line write-on reclosable small parts bags, 3 x 5; part # 47235. <http://www.c-lineproducts.com/qz375-write-on-small-parts-bags-3-x-5-47235-cli47235-cli-47235.html>

## H. HAIR COLLECTION PROCEDURE

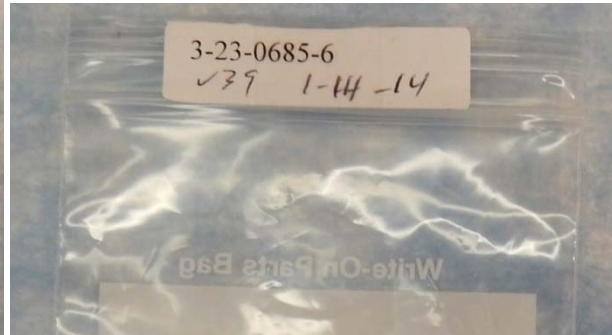
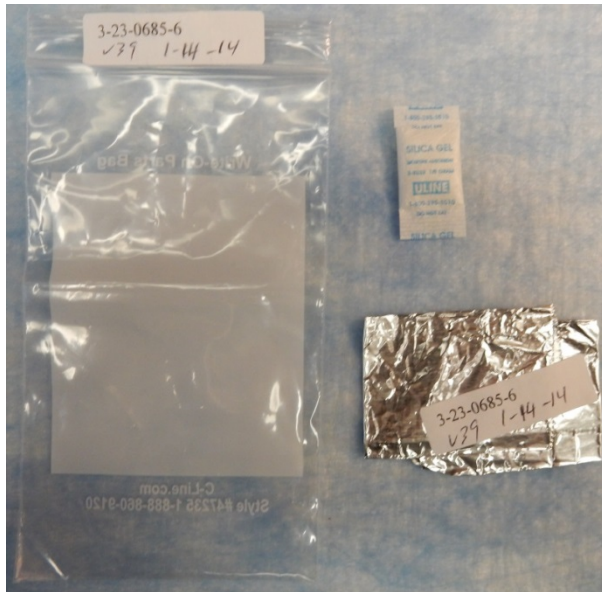
Clinicians at each site will collect the hair sample during a core visit on each HIV-positive participant who has taken ARV medications since her last study visit. If the participant is HIV-negative or HIV-positive and not on antiretroviral medications, “Not Applicable” should be noted on the *Specimen Collection Form (F31/F31r)*. It is recommended that hair samples be collected during the physical exam. A series of pictures illustrating the hair collection procedure on different hair types are provided below. A video illustrating the collection of 30-40 strands of hair can be found at the

following link: <http://www.youtube.com/user/lshaanvideos#p/a/u/0/-T8ltDH7xbl>. A video illustrating the collection of 100 strands of hair can be found at the following link: <http://www.youtube.com/watch?v=F1Fd0b2llaQ>. A video produced for participants that explains the hair collection process and why we collect hair samples in the WIHS (along with providing a participant perspective) can be viewed on the WIHS web site: <http://statepiaps.jhsph.edu/wihs/admin/clinical-training/clinical-training.htm>.

1. Clean the blades of a pair of scissors with an alcohol pad and allow blades to completely dry prior to use.
2. Unfold the piece of aluminum foil and have it ready, along with a small label for labeling the hair once cut.
3. 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 from *underneath* this top layer of hair from the **occipital** region.
4. Women on tenofovir-based regimens should be asked if they are willing to have 100 fibers of hair collected. The participant should be given an option to OPT OUT from the collection of 100 strands even if she is on tenofovir (drug code 234, 253, 262, 280, 287) and default to the routine pattern of collecting 30-40 strands.
5. For women on tenofovir who agree to the 100 strands of hair collection, the hair can be collected from more than one spot in the back of the head if this increases acceptability.
6. For women who are not on tenofovir-based regimens, 30-40 fibers of hair should be isolated and cut.
7. Cut the small hair sample off the participant's head *as close to the scalp as possible*.
8. Lay the small hair sample onto the piece of unfolded aluminum foil and place a small label with the participant's WIHSID over the **distal** end of the hair thatch (affixing the hair sample to the tin foil in the process). 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.
9. Refold the foil over to completely enclose the thatch of hair.
10. Prepare two labels with specimen ID (containing WIHSID, Visit number, and Date specimen collected) for each specimen. The WIHSID should be formatted to include dashes, e.g., WIHSID 32306866 is formatted as 3-23-0686-6. An example of the requested label format is below:



11. Place the Ziplock bag reverse side up (with the write-on section facing down). Place the specimen ID label on the top by the seal. The label should not over-hang the top edge of the bag.



12. Place the specimen and desiccant in the Ziplock bag. The label on the foil must face up. Push the foil-wrapped specimen and the desiccant to the bottom of the bag.



13. Fold the bag in half and secure with tape. The specimen ID will be visible on both sides of the folded bag. Use a small piece of tape placed on the side of the seal to secure the fold.



14. 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.
15. Hair samples should be kept at room temperature and in a dark place at each site prior to shipment.



## I. 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.



Indestructo Mailers 50/bundle

Vendor	Phone	Cat. #	price
<b>PackagingPrice.com</b>	888-236-1729	MLR84	29.50
<b>The Box Depot</b> 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 Bradley Aouizerat at [bea4@nyu.edu](mailto:bea4@nyu.edu) (with copy to Ratna Veeramachaneni at [rv48@nyu.edu](mailto:rv48@nyu.edu)) on the day of shipment along with the shipment tracking number:

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

- The WIHS Hair Repository is to be notified of each shipment by calling 917-821-6034. 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:

WIHS Hair Repository  
 c/o Ratna Veeramachaneni  
 Dr. Bradley Aouizerat  
 NYUCD  
 345 East 24<sup>th</sup> Street (Lab Room 1011)  
 New York, NY 10010  
[Rv48@nyu.edu](mailto:Rv48@nyu.edu)  
 917-821-6034

References:

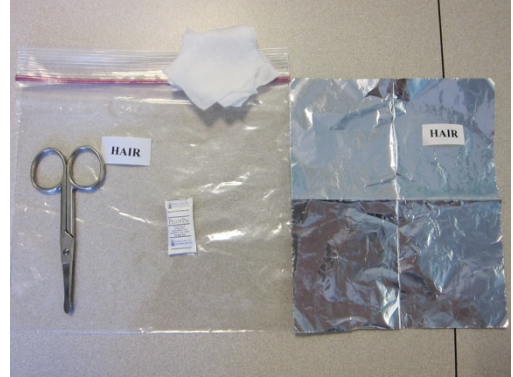
1. Podsadecki TJ, Vrijens BC, Tousset EP, Rode RA, Hanna GJ. "White coat compliance" limits the reliability of therapeutic drug monitoring in HIV-1-infected patients. *HIV Clin Trials* **2008**; 9:238-46.
2. Lescure FX, Poirier JM, Meynard JL, et al. Factors predictive of virological failure on atazanavir in 310 HIV-infected patients. *Aids*; 24:1593-5.
3. Ray JE, Marriott D, Bloch MT, McLachlan AJ. Therapeutic drug monitoring of atazanavir: surveillance of pharmacotherapy in the clinic. *Br J Clin Pharmacol* **2005**; 60:291-9.
4. Gandhi M, Ameli N, Bacchetti P, et al. Atazanavir concentration in hair is the strongest predictor of outcomes on antiretroviral therapy. *Clin Infect Dis* **2011**; 52:1267-75.
5. Gandhi M, Ameli N, Bacchetti P, et al. Protease inhibitor levels in hair strongly predict virologic response to treatment. *AIDS* **2009**; 23:471-8.
6. Gandhi M, Greenblatt RM, Bacchetti P, et al. A Single-Nucleotide Polymorphism in CYP2B6 Leads to >3-Fold Increases in Efavirenz Concentrations in Plasma and Hair Among HIV-Infected Women. *J Infect Dis* **2012**; 206:1453-1461.
7. Huang Y, Gandhi M, Greenblatt RM, Gee W, Lin ET, Messenkoff N. Sensitive analysis of anti-HIV drugs, efavirenz, lopinavir and ritonavir, in human hair by liquid chromatography coupled with tandem mass spectrometry. *Rapid Commun Mass Spectrom* **2008**; 22:3401-9.
8. Huang Y, Yang Q, Yoon K, et al. Microanalysis of the antiretroviral nevirapine in human hair from HIV-infected patients by liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem* **2011**; 401:1923-33.
9. van Zyl GU, van Mens TE, McIlleron H, et al. Low lopinavir plasma or hair concentrations explain second-line protease inhibitor failures in a resource-limited setting. *J Acquir Immune Defic Syndr* **2011**; 56:333-9.
10. Liu A, Yang Q, Huang Y, et al. Dose Proportionality of Tenofovir in Hair in HIV-Negative Men and Women: A Novel Method of Monitoring Adherence to Pre-Exposure Prophylaxis (PrEP). **2013** (submitted).
11. Gandhi M, Mwesigwa J, Aweeka F, et al. Hair and Plasma Data Show that Lopinavir, Ritonavir and Efavirenz All Transfer from Mother to Infant in Utero, but only Efavirenz Transfers via Breastfeeding. *JAIDS* (in press) **2013**.



## WIHS V- Hair collection pictures

**Materials required:** Scissors, piece of tin foil, patient labels (2), ziplock bag, alcohol swabs, desiccant

*Suggest making these "hair kits" ahead of time*



**Step 1:** Clean the blades of a pair of scissors with an alcohol pad and allow blades to completely dry

*Clean off blades of scissors between patients*



**Step 2:** Lift up the top layer of hair from the occipital region of the scalp. Isolate a small thatch of hair (100 fibers of hair for participants on tenofovir-based regimens; 30-40 strands for women who are not on tenofovir-based regimens) from underneath this top layer

*Can use hair clip to keep top layer of hair away if easier*

**Step 3:** Cut the small hair sample as close to the scalp as possible

### STRAIGHT HAIR



### CURLY HAIR



## SHORT HAIR

*Can let hair fall directly into piece of tin foil when very short/cropped (no need to label end)*



## BRAIDED HAIR or DREADS

*Cut hair thatch from in-between braids or dread locks*



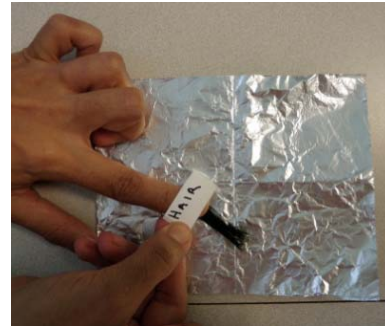


**Step 4:** Keep your fingers on the part of the hair that was FURTHEST away from the scalp and put the hair sample down on an unfolded piece of tin foil



**Step 5:** Put a thin label over the end of the hair sample that was FURTHEST away from the scalp

*If hair very short, just let it fall into the piece of tin foil and no need to label the distal end*



**Step 6:** Refold the foil over to completely enclose the hair and place a study ID label on the folded piece of foil



**Step 7:** Place the folded piece of foil inside the plastic (e.g., Ziplock®) bag with desiccant and seal the bag



Hair samples should be kept at room temperature and in a dark place at each site prior to batch shipment (without biohazardous restrictions) to the WIHS Hair Repository at NYU.