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.

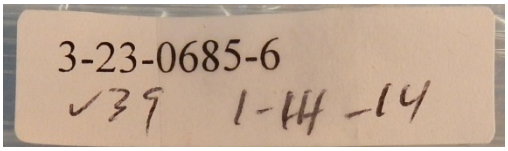
- Aluminum foil can be ordered from **Quill Diagnostics**. The product is called **Handy Foil Standard Aluminum Foil**, catalogue number 035-11205: 12 inches x 100 feet, $39.99. Alternatively, the foil can be purchased locally, if sites can find a better price. Aluminum foil should be cut into squares 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

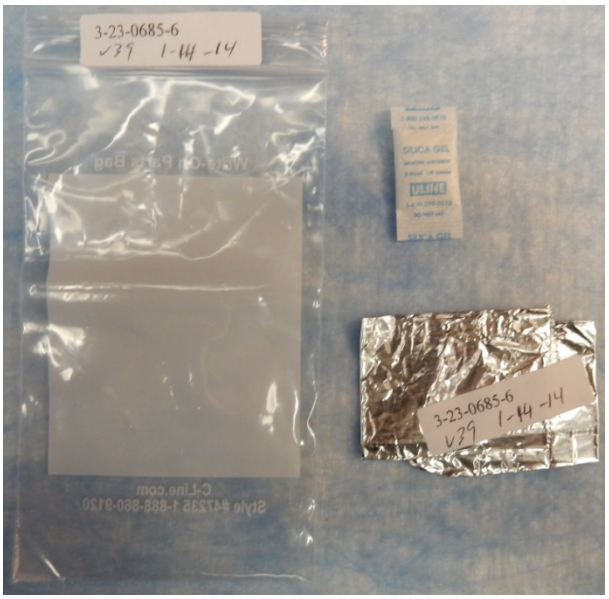
Clinicians at each site will collect the hair sample during a core visit on each HIV-positive participant who has taken ARV medications since her last study visit. If the participant is HIV-negative or HIV-positive and not on antiretroviral medications, "Not Applicable" should be noted on the *Specimen 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

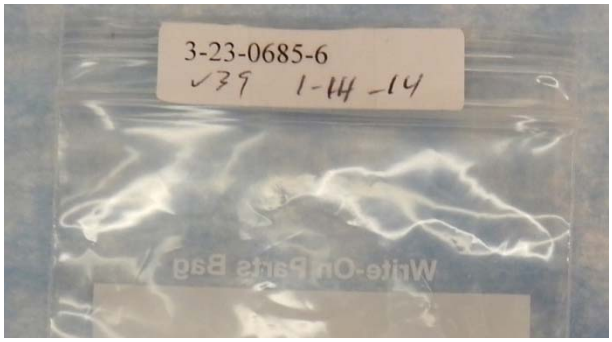
following link: http://www.youtube.com/user/Ishaanvideos#p/a/u/0/-T8ItDH7xbI. A video illustrating the collection of 100 strands of hair can be found at the following link: http://www.youtube.com/watch?v=F1Fd0b2IlaQ. A video produced for participants that explains the hair collection process and why we collect hair samples in the WIHS (along with providing a participant perspective) can be viewed on the WIHS web site: https://statepi.jhsph.edu/wihs/admin/clinical-training/.

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 ahead *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:



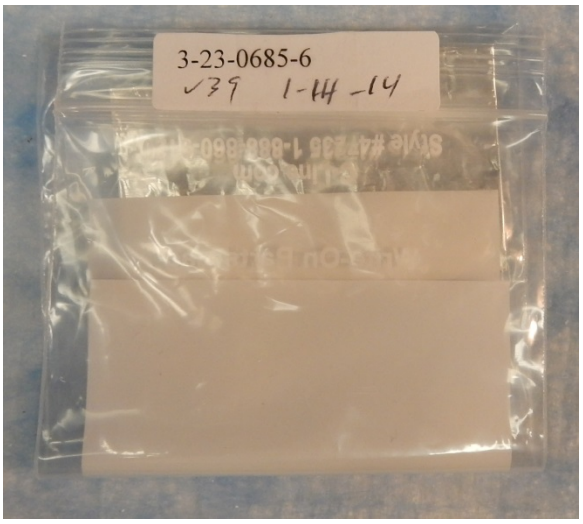
11. Place the Ziplock bag reverse side up (with the write-on section facing down). Place the specimen ID label on the top by the seal. The label should not over-hang the top edge of the bag.

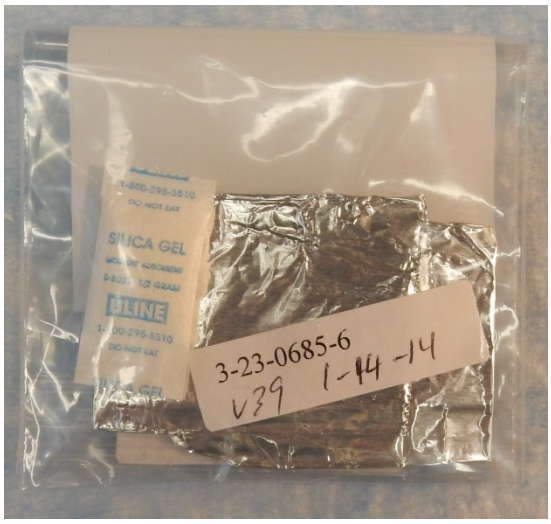


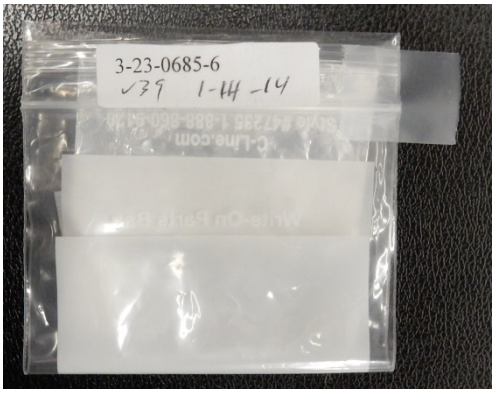


12. Place the specimen and desiccant in the Ziplock bag. The label on the foil must face up. Push the foil-wrapped specimen and the desiccant to the bottom of the bag.

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
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 Microsoft Excel file in the following format must be e-mailed to the WIHS Hair Repository at WIHS_hair@nyu.edu and copy Bradley Aouizerat, MS, PhD (bea4@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 1-212-998-9203. 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 Ronald Lam, MS
Dr. Bradley Aouizerat
New York University College of Dentistry
345 East 24th Street (Lab Room 1008S)
New York, NY 10010

Email: WIHS_hair@nyu.edu
Laboratory telephone number: 1-212-998-9203

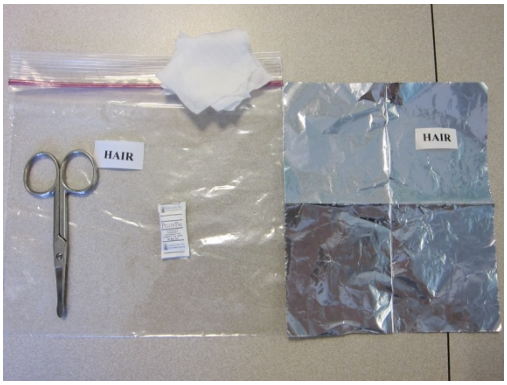
References:

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

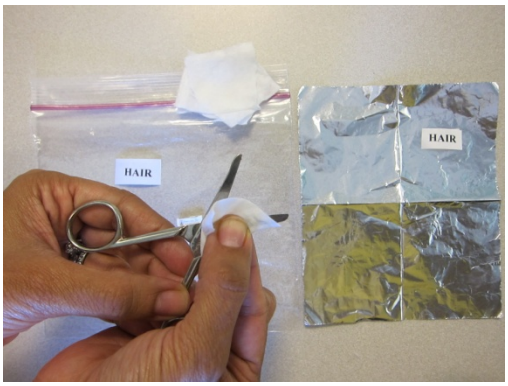
Materials required: Scissors, piece of tin foil, patient labels (2), ziplock bag, alcohol swabs, desiccant

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)

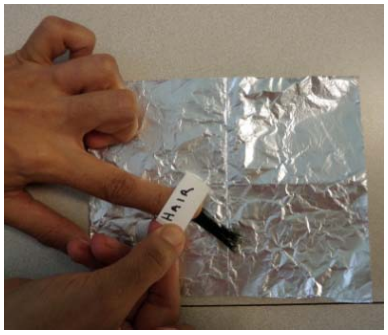
BRAIDED HAIR or DREADS

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.

APPENDIX A:

Use of Hair Concentrations in the Women's Interagency HIV Study:

Statistical Notes

Examples of Previously Conducted Analyses

This brief guide, along with a database of antiretroviral hair concentrations and its accompanying data key, are provided for investigators who may be interested in using these hair concentrations in various analyses, with specific guidance on data analysis and practical considerations.

Investigators are encouraged to consult the listed content experts for additional assistance in proposing concept sheets and planning analyses involving hair pharmacology.

Included below is a list of the relevant research publications/presentations from the WIHS to date (July 27, 2018).

PUBLISHED WORK

1. Tamraz B, Huang Y, French AL, Kassaye S, Anastos K, Nowicki MJ, Gange S, Gustafson DR, Bacchetti P, Greenblatt RM, Hysi PG, Aouizerat BE. A genome-wide association study identifies a candidate gene associated with atazanavir exposure measured in **hair**. Clin Pharmacol Ther. 2018 Jan 9. doi: 10.1002/cpt.1014.
2. Baxi SM, Greenblatt RM, Bacchetti P, Jin C, French AL, Keller MJ, Augenbraun MH, Gange SJ, Liu C, Mack WJ, Gandhi M; Women's Interagency HIV Study (WIHS). Nevirapine Concentration in **Hair** Samples Is a Strong Predictor of Virologic Suppression in a Prospective Cohort of HIV-Infected Patients. PLoS One. 2015 Jun 8;10(6):e0129100. doi: 10.1371/journal.pone.0129100. eCollection 2015.
3. Gandhi M, Greenblatt RM, Bacchetti P, Jin C, Huang Y, Anastos K, Cohen M, Dehovitz JA, Sharp GB, Gange SJ, Liu C, Hanson SC, Aouizerat B; Women's Interagency HIV Study. 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 Nov;206(9):1453-61.
4. Gandhi M, Ameli N, Bacchetti P, Anastos K, Gange SJ, Minkoff H, Young M, Milam J, Cohen MH, Sharp GB, Huang Y, Greenblatt RM. Atazanavir concentration in **hair** is the strongest predictor of outcomes on antiretroviral therapy. Clin Infect Dis. 2011 May;52(10):1267-75.
5. Gandhi M, Ameli N, Bacchetti P, Gange SJ, Anastos K, Levine A, Hyman CL, Cohen M, Young M, Huang Y, Greenblatt RM; Women's Interagency HIV Study (WIHS). Protease inhibitor levels in **hair** strongly predict virologic response to treatment. AIDS. 2009 Feb 20;23(4):471-8.

CONFERENCE PRESENTATIONS

1. Dickinson L , Siccardi M, Anastos K, Cohen MH, Gustafson D, Sharp GB, Gange SJ, Kebede S, Bacchetti P, Greenblatt RM. Efavirenz Level in Hair Predicts Virologic Response Better Than Level in Blood. CROI 2018. Abstract 462.

2. Alfredo J. Aguirre, C. Christina Mehta, Sophia A. Hussen, Ighovwerha Ofotokun, Ruth M. Greenblatt, Daniel Merenstein, Angela D. Kashuba, Kathryn Anastos, Mirjam-Colette Kempf, Elizabeth T. Golub, Jack A. Dehovitz, Mardge H. Cohen, Sheri Weiser, Anandi N. Sheth. Low-Level Viremia is Associated with Lower Protease Inhibitor Levels in Hair. CROI 2018. Abstract 461/

3. Anna M. Leddy, Lila A. Sheira, Bani Tamraz, Tracey E. Wilson, Adebola Adedimeji, Daniel Merenstein, Mardge H. Cohen, Eryka L. Wentz, Adaora A. Adimora, Ighovwerha Ofotokun, Lisa Metsch, Janet M. Turan, Peter Bacchetti, Sheri D. Weiser. Food insecurity is associated with lower levels of antiretroviral drug concentrations in hair among a cohort of women living with HIV in the United States. IAPAC Adherence Conference. Abstract 216.

I. Notes on statistical analysis of drug concentrations in hair

I assume that the distributed data set will be in long format (one observation per drug per visit per woman) and will have the following variables:
WIHSID
VISIT
ARV
ARV_HAIR
SPECIMEN_LLOQ (the specimen-specific LLOQ)

Below Limit of Quantification (BLQ) values

When ARV_HAIR = -777, this indicates that the concentration of the ARV in the hair specimen was below the lower limit of quantification (LLOQ) for the assay. This varies from specimen to specimen due to the amount of hair assayed (and possibly other factors), and its value is given by SPECIMEN_LLOQ. Because the assay is very sensitive, these LLOQ's are usually very small, around 1% of the median observed values of ARV_HAIR. For many purposes, distinctions between very low hair concentrations will not be important, but differences between very low values can become very large when logarithmically transformed (see next section). One way to prevent this is to limit how small ARV_HAIR is allowed to be, by resetting any values below a specified limit (including BLQ values) to instead equal the limit. This is akin to the statistical procedure known as Winsorizing. Because different ARVs have different concentrations in hair, the specified limit should be different for different ARVs. For each ARV, the specified limit could be defined in various ways, such as: a) The largest SPECIMEN_LLOQ among all observations with ARV_HAIR = -777 for that ARV; b) The largest SPECIMEN_LLOQ among all observations for that ARV; c) 2% of the median observed ARV_HAIR for that ARV. Limit a) is the smallest possibility that will clear specify what to do with all BLQ values; therefore, if limit c) is less than

a), then it should not be used for that ARV. Whatever limit is chosen, I recommend creating a variable WinsorValue that is equal to this value for each observation. This will make the analysis data set more self-documenting. Also helpful is to give a new name to the resulting variable, such as ARV_HAIRw.

Another way to handle BLQ values is to use *single imputation*. Two common choices are to reset ARV_HAIR = SPECIMEN_LLOQ or to reset ARV_HAIR = SPECIMEN_LLOQ/2 when it is -777 (BLQ). This is similar to the "Winsorizing" approach above with limit a), except that it can impute different values for different BLQ specimens. For many purposes, it may be undesirable to treat different BLQ specimens as having different ARV concentrations just because of the amount of hair assayed.

Another possibility is to treat BLQ specimens as left-censored observations of the ARV concentration, meaning that the statistical analysis will only use the information that the true ARV concentration is some value less than SPECIMEN_LLOQ. Although this most accurately reflects the information provided by the assay, it requires an additional layer of complexity in the statistical analysis, and the information that is preserved by this approach may not be meaningful for many purposes (as noted above). This approach would therefore mainly be worthwhile when distinctions between very small values are meaningful for the issue being investigated. For example, a study of biological influences on long-term pharmacokinetics and/or dynamics of ARV incorporation into hair, restricted to persons known to have been fully adherent, might benefit from this approach. To implement this approach, we set ARV_HAIR = SPECIMEN_LLOQ and LeftCensor =1 for observations with ARV_HAIR = -777, and we set LeftCensor =0 for all other observations.

Logarithmic transformation

ARV concentrations in hair tend to be right-skewed. In addition, they are intended to measure ARV *exposure*, which is usually logarithmically transformed for pharmacokinetic analyses. For most purposes, hair concentrations should therefore be logarithmically transformed before performing statistical analyses. Note, however, that log transformation may give too much importance to differences between very low concentrations. For example, the numerical difference between a concentration at the LLOQ and one that is twice the LLOQ will be the same as the difference between the median concentration and twice the median. For many purposes, the former may be less important than the latter, and the numerical differences can be made to reflect this by using a modified transformation:
logHair = log(*c* + ARV_HAIR)
where *c* is a small constant, such one of the limits a), b), or c) discussed above for the "Winsorizing" approach:
logHairw = log(WinsorValue + ARV_HAIRw)
Ideally the constant added will be small enough that back-transformed coefficients can still be interpreted as approximately multiplicative effects over most of the range of hair concentration values, while also large enough to appropriately downweight differences between very small values. For projects that treat BLQ values as left-censored, no modification should be used (*c*=0).

For analyses where hair concentrations are an outcome variable, the base of the logarithm used does not matter, because coefficients will be back-transformed into multiplicative effects:
Base 10: fold-effect = $10^{coefficient}$
Base 2: fold effect = $2^{coefficient}$
Natural log: fold-effect = $e^{coefficient}$ = exp(coefficient)
Note that in Stata, use of natural log enables use of the "eform" option on some commands to conveniently obtain back-transformed effects.

If using hair concentration as a predictor variable, then use of log base 2 or log base 10 will usually be a good choice, because these make interpretation easier. For example, using log base 2 results in regression models that estimate the effect on the outcome per 2-fold difference in hair concentration. For example:
log2Hairw = log(WinsorValue + ARV_HAIRw)/log(2)

Dichotomization

For many purposes, dichotomizing hair concentrations would throw away too much valuable information, so they should usually be analyzed as continuous variables. One dichotomization that has been done for a specialized purpose was based on whether a logistic regression model predicted >50% chance of a detectable viral load based on the hair concentration of each particular ARV. If you believe that you have such a specialized purpose, I recommend consulting a skeptical statistician before reducing hair concentrations to a yes/no variable.

Example analyses of predicting hair concentrations of a particular ARV, using visits where participants reported taking the ARV

SAS, "Winsorizing" approach:

```
proc mixed noclprint noitprint covtest;
  class wihsID;
  model logHairw = pred1 pred2 / s cl;
  random int / subject=wihsID;
Run;
```

Stata, "Winsorizing" approach:

```
mixed logHairw pred1 pred2 || wihsID:
```

SAS, left-censored approach:

```
proc nlmixed;
  parms int=0 b1=0 b2=0 sig=0.75  reSD=0.75; * starting values - other choices may
work better ;
  sigsq = sig*sig; * residual variance ;
  pi=2*arsin(1);
  mu=   u + int + b1*pred1 + b2*pred2; * the regression model ;
  if Censored=0 then L = (1/(sqrt(2*pi*sigsq)))*exp(-(logHair-mu)**2/(2*sigsq)); *
detectable concentration;
  if Censored=1 then L = probnorm((logHair - mu)/sqrt(sigsq)); * BLQ ;
  ll = log(L); * the log-likelihood when logHair is normally distributed ;
  model logHair ~ general(ll);
  random u ~ normal(0, reSD*reSD) subject=ID; * a random effect accounting for within-
participant correlation;
run;
```

```
/*
Reference: Thiebaut R, Jacqmin-Gadda H. Mixed models for longitudinal left-censored
repeated measures.
           Comput Methods Programs Biomed. 2004 JUN;74(3):255-60.
*/
```

Stata, left-censored approach:

```
gen lowerLimit=logHair if censored==0
meintreg lowerLimit logHair pred1 pred2 || wihsID:
```

Combined analysis of hair concentrations of multiple ARVs

When modeling factors that influence hair concentrations, we may sometimes expect some or all factors to have similar influences on different ARVs. In this case, we can better estimate a common effect of the predictor(s) by using a model that includes all the observations on all the ARVs of interest. (A reasonable "due-diligence" assessment of the assumption that a predictor has a similar influence on hair concentrations of the different ARVs can be performed by adding a term for predictor by ARV interaction to the model.) Depending on the ARVs of interest for a particular study question, some participants may have measures of two or more ARVs at the same visit, so the example code below allows for this by using hierarchical random effects modeling. (As noted above, the distributed data set is assumed to be in the "long" form that will facilitate this type of analysis.) If there is only one ARV measured at every visit, then omit the second level of random effects.

SAS, "Winsorizing" approach:

```
proc mixed noclprint noitprint covtest;
  class wihsID visit ARV;
  model logHairw = ARV pred1 pred2 / s cl; * Including ARV allows for
differing means;
  random int / subject=wihsID;
  random int / subject=visit(wihsID); * ARVs measured at the same visit may
be correlated;
  repeated / group=ARV; * this allows the residual variance to differ for
different ARVs;
Run;
```

Stata, "Winsorizing" approach:

```
mixed logHairw i.ARV pred1 pred2 || wihsID: || visit: ,
residuals(independent, by(ARV))
```

For the left-censored approach, the meintreg command may work with the additional random effect level and option:

```
meintreg lowerLimit logHair pred1 pred2 || wihsID: || visit: ,
residuals(independent, by(ARV))
```

As far as I know, however, this has not yet been tried by WIHS investigators.

The left-censored approach for this situation may not be possible in SAS.

Some factors may have similar effects on some ARVs and no effect on others. For example, kidney function may influence all the ARVs that are removed by the kidney but have no effect on ARVs that are not. So we could model this with a modified predictor:
eGFRcPer10 = (eGFR – 100)/10 for ARVs that are removed by the kidney
= 0 for ARVs that are not
Here, the centering at 100 is so that the non-kidney ARVs are compared to the other ARVs when participants have normal kidney function, and the re-scaling to make the results more interpretable (1 point of eGFR is very small).

Using hair concentrations of multiple ARVs to define a single predictor

For some issues, it may be reasonable to assume that hair concentrations of different ARVs that are comparable in some sense will have a similar effect on an outcome of interest, such as a detectable viral load. As far as I know, this has not yet been done in WIHS, but there are a number of possibilities, all of which are somewhat ad hoc:

1. Calculate Z-scores for each ARV. For visits with more than one ARV measured in hair, average the Z-scores to create the single predictor.
2. Categorize concentrations of each ARV (e.g., into quartiles). For visits with multiple ARVs, use the higher category for the single predictor.
3. Calculate how far each ARV concentration is above or below the median, on the log2 scale. For visits with more than one ARV measured, average as for Z-scores.

Use of such a predictor requires assumptions that may not hold. For example, Z-scores of -1 and +1 may have similar risk of detectable viral load for an ARV that has a wide therapeutic range, while the risks may differ greatly for a different ARV. Therefore, performing separate analyses for different ARVs may usually be preferable, and this is what has generally been done in WIHS.

Content Expert: Peter Bacchetti, UCSF WIHS (in phased retirement, limited availability), Ruth Greenblatt, UCSF WIHS (in phased retirement, limited availability)

II. EXAMPLES OF DATA ANALYSIS USING HAIR DATA IN THE WIHS

Hair levels and self-reported adherence discrepancies in WIHS

Many published studies support the association between various measures of adherence including self-report, hair, dried blood spot, and electronic monitors. Further published literature supports the relationship between these individual measures and their correlation with HIV viral load or viral load reduction. Hair levels are often incorporated into WIHS studies as the more "objective" measure of long-term medication adherence. However, there is a small proportion of WIHS participants who have discrepancies between the values of antiretrovirals measured in hair and self-report. In our work, we have examined groups which outline these discrepancies: self-reported adherence < 75% and high levels of ART In hair; also high self-reported adherence > 95% and low levels of ART in hair and found that they include a small proportion of the population (~12%). These discrepancies may have many potential origins. Biological factors much as impaired kidney or liver function, body composition, or drug-drug interactions may impede (or facilitate) higher levels of ART in hair. Other psychosocial factors may affect the memory around number of ingested doses, and other psychosocial factors may lend themselves to social desirability reporting during visits.

This may be important for researchers to consider as they determine the purpose of a selected adherence predictors for their model. For example, if the goal is to assess *the effect of intended doses* on a particular outcome, self-reported adherence would reflect this most closely. If it is more important to assess *the impact of absorbed/actual exposure* to antiretrovirals, then a hair level may be preferred. Another approach may be to use one or the other, but conduct a type of sensitivity check to determine the proportion of discrepancies and report on any implications of this.

Content expert: Jennifer Cocohoba, UCSF WIHS, Ruth Greenblatt, UCSF WIHS (in phased retirement, limited availability)

Genome-Wide Association Studies and Antiretroviral Hair Exposure

Detailed methods are described in this paper.

Full Reference: Bani Tamraz, Yong Huang, Audrey L. French, Seble Kassaye, Kathryn Anastos, Marek J. Nowicki, Stephen Gange , Deborah R. Gustafson, Peter Bacchetti, Ruth M. Greenblatt, Pirro G. Hysi, Bradley E. Aouizerat. A Genome-Wide Association Study Identifies a Candidate Gene Associated With Atazanavir Exposure Measured in Hair. 2018 Jan 9. doi: 10.1002/cpt.1014. [Epub ahead of print]

Content experts: Bani Tamraz, UCSF WIHS; Brad Aouizerat, UCSF WIHS

APPENDIX B

Hair Database Notes
Lila Sheira
February 8th, 2018

I removed the following variables from the analytic database:

- Dose_Route : all values were n/a
- Dose_Time : all values were n/a
- Time_Sample_Collection : all values were n/a
- Treatment_group: all values were n/a
- PK_Timepoint: all values were n/a
- Took out infectious organism column as all values were HIV
- Took out matrix as all values said hair
- Took out species as all were human

Changes to avoid importing as string variables:

- Changed insufficient volume to -999
- Changed BLQ to -777 (for now, later recoded, see below)
- Changed N/A to -555
- Renamed column "Analytes requested" to "DRUGCLASS" and categorized drug classifications:
    - ATZ, DRV: 1
    - ATZ, DTG: 2
    - ATZ, RAL: 3
    - DRV: 4
    - DRV, DTG: 5
    - DRV, RAL: 6
    - DTG: 7
    - RAL: 8
    - ATZ: 9
- Changes all the following variables so that they were shorter:

Excel name	New Stata name
ATV_Analyte_Concentration_ngmL	ATV_Analyte_ngml
ATV_Analyte_Concentration_ngm	ATV_ngmg_hair
ATV_Sample_Specific_LLOQ_ngmg	ATV_LLOQ_ngmg
RAL_Analyte_Concentration_ngmL	RAL_Analyte_ngmL
RAL_Analyte_Concentration_ngmg	RAL _ngmg _hair
RAL_Sample_SpecificLLOQ_ngmgh	RAL_LLOQ_ngmg
DTG_Analyte_Concentration_ngmL	DTG_Analyte _ngmL
DTG_Analyte_Concentration_ngmg	DTG_ngmg_hair
DTG_Sample_Specific_LLOQ_ngmg_h	DTG_LLOQ_ngmg
DTG_Metabolite_Peak_Area	DTG_Metabolite_Peak_Area
DTG_Metabolite_Concentration_Pea	DTG_Metabolite_Concentration_Peak
DTG_Percent_Peak_Area_Metabolite	DTG_Percent_Peak_Area_Metabolite
DRV_Analyte_Concentration_ngmL	DRV_Analyte_ngmL

DRV_Analyte_Concentration_ngmg	DRV_ngmg_hair
DRV_SampleSpecific_LLOQ_ngmg	DRV_ LLOQ_ngmg

- After conversations with Peter and the UNC hair team, we decided to recode values BLQ to be at the limit of quantification. An alternative proposed by UNC was ½ the LLOQ but we opted not to do that. These are the values originally coded as -777. Here is the code:
    - recode DTG_ngmg_hair (-777=.0046729) if WIHSID==12120825 & VISIT==42
    - recode DTG_ngmg_hair (-777=.00353607) if WIHSID==30100272 & VISIT==40
    - recode DTG_ngmg_hair (-777=.00338983) if WIHSID==81400421 & VISIT==39
    - recode DTG_ngmg_hair (-777=.00441501) if WIHSID==81401839 & VISIT==42
    - recode DTG_ngmg_hair (-777=.00314861) if WIHSID==83411185 & VISIT==41
        - NB: The hair is important to recode; not so much the analyte concentration which is still coded as -777.
- For women on combination therapies at one visit, they have two rows of results which are classified as ATZ, DRV (for example) and one row has values only for ATZ and the other for DRV, although both classified as combinations. To correct this and have one label per person-visit-drug, I did the following two steps:
    - I made a generic hair value regardless of drug classification by making a new variable that sums the four hair results per row. Since for all women 3 of the 4 are missing, it only sums the present value (stata code below)
        - egen ARV_HAIR= rowtotal(ATV_ngmg_hair RAL_ngmg_hair DTG_ngmg_hair DRV_ngmg_hair) if DTG_ngmg_hair>0
        - gen logARV_HAIR=log(ARV_HAIR)
    - I then made 4 indicator variables by row to see which drug class there was by row using the following code (the fact that it is a mean doesn't matter; just trying to "tag" present values for each class):
        - egen ATV_class=rowmean(ATV_Analyte_ngml ATV_ngmg_hair ATV_LLOQ_ngmg)
        - egen DRV_class=rowmean(DRV_Analyte_ngmL DRV_ngmg_hair DRV_LLOQ_ngmg)
        - egen DTG_class=rowmean(DTG_Analyte_ngmL DTG_ngmg_hair DTG_LLOQ_ngmg)
        - egen RAL_class=rowmean(RAL_Analyte_ngmL RAL_ngmg_hair RAL_LLOQ_ngmg)
    - I then used these new variables to code a new drug class variable
        - gen DRUGCLASS2=.
        - replace DRUGCLASS2=1 if ATV_class!=.
        - replace DRUGCLASS2=2 if DRV_class!=.
        - replace DRUGCLASS2=3 if DTG_class!=.
        - replace DRUGCLASS2=4 if RAL_class!=.
        - label define DRUGCLASS2 1 "ATZ" 2 "DRV" 3 "DTG" 4 "RAL"
        - label values DRUGCLASS2 DRUGCLASS2
        - rename DRUGCLASS2 DRUGCLASS
    - To check my work I ran the following:

- list WIHSID VISIT DRUGCLASS DRUGCLASS2 ARV_HAIR ATV_ngmg_hair ATV_class RAL_ngmg_hair RAL_class DTG_ngmg_hair DTG_class DRV_ngmg_hair DRV_class in 1/75
- I checked that 1) ARV hair level matches the level for whichever drug class is per that level and 2) the label matches the individual (not combination) therapy for that level.