WOMEN'S INTERAGENCY HIV STUDY

SECTION 15: NIDA IMMUNOLOGY/VIROLOGY SUBSTUDY PROTOCOL

A. STUDY PURPOSE

The Women's Interagency HIV Study (WIHS) Immunology/Virology Substudy is funded by a grant from NIDA to study four issues related to drug use that may affect the course of HIV-1 infection and disease progression. These issues are:

1. route of infection, that is mucosal vs. parenteral infection;
2. antigenic and immunologic stimulation in the infected individual due to exposure to street drugs;
3. number of sexual partners, which is related to sexually transmitted diseases and antigenic stimulation; and
4. adherence to regimens of combination antiretroviral therapy which will affect HIV-1 viral load and disease progression – an issue which arises now that there are powerful drugs available to treat HIV-1.

B. RESEARCH AIMS

To perform serial virologic, molecular, and immunologic studies on the WIHS NIDA Virology and Immunology cohort. The specific aims are:

1. To obtain specific additional information to document use of street drugs and antiretroviral drugs.
2. To quantitate and compare HIV-1 viral load in two body compartments. To determine HIV-1 RNA loads in plasma as part of the core WIHS protocol and HIV-1 RNA loads in the female genital tract as part of this study.
3. To study the degree of immune response and activation by performing immunophenotypic studies including 3-color flow cytometric evaluation of peripheral blood cells for markers of activation (HLADR, CD38), maturation (CD45RO, CD62L), and function (CD28, CD95).

In addition, intensive laboratory studies will be performed on a group of 56 participants selected to represent each category of drug use, number of sexual partners, and those with high and low viral loads. In these participants, the aims are:

1. To perform quantitative culture of HIV-1 from the peripheral blood (peripheral blood mononuclear cells [PBMCs]) and genital tract (cervicovaginal lavage [CVL]).
2. To characterize the virus in these participants by determining the cellular phenotype, and in some cases, cell tropism of infectious virus isolated from PBMC and CVL.
3. To compare the degree and evolution of HIV-1 sequence diversity found in infected women who use street drugs and those who do not and the levels of diversity in different body reservoirs of HIV-1 by determining the RNA sequence of the hypervariable V1-V5 portion of the HIV-1 <u>env</u> gene from virus in plasma and contemporaneous CVL; in selected cases the sequences of complete HIV-1 RNA genomes derived from plasma and CVL will be compared.
4. To study the effect of antiretroviral therapy on mutations conferring antiviral resistance by determining the nucleic acid sequence of the HIV-1 <u>pol</u> gene from blood and CVL of women with different patterns of antiretroviral and street drug use.
5. To evaluate cytokine regulatory pathways by measuring proinflammatory and immunoregulatory cytokines and chemokines in blood and CVL samples following <u>in vitro</u> stimulation.

6. For overall analyses, results of viral and immunologic analyses of the blood will be correlated with each other and with data obtained from contemporaneous CVL samples of the same individuals. In addition, data will be analyzed to study virology, immunology, and clinical HIV-1-related disease as they relate to pattern of street drug use, route of infection, antigenic and immune stimulation, and antiretroviral therapy.

C. BACKGROUND

The course of HIV-1 infection and disease progression in infected individuals is marked by great variability. Virologic and immunologic factors including HIV-1 load, nucleotide sequence, viral phenotype, humoral and cellular response, antiviral resistance, and viral reservoirs are relevant to the control of HIV-1 infection. We currently plan to test the following hypotheses related to drug use in women:

1. The course of HIV-1 infection, as measured by clinical disease progression and virologic and immunologic parameters in the blood and genital tract, differs in women infected mucosally by sexual transmission as compared with those infected parenterally by injection of drugs.

2. The course of infection may differ in women who are continuously exposed to the antigenic stimulation and immune activation of injecting or non-injecting drug use as compared to women who have not used these substances. In addition, antigenic and immunologic stimulation may be due to sexually transmitted diseases associated with drug use and multiple sexual partners.

The long-term effects of route of HIV-1 infection and immunologic stimulation also relate to longstanding fundamental questions of HIV-1 pathogenesis in infected individuals. We have designed a study that takes advantage of the unique resources of the WIHS cohort to answer these fundamental, clinically relevant questions. The detailed information in the WIHS database related to drug use, sexual history, and immunologic status enabled us to select a broad spectrum of individuals in whom we can investigate these scientific hypotheses. This study promises to break ground in three significant ways:

1. It will be a large, well-controlled study of the relationship of drug use to the course of HIV-1 infection and disease progression in women.

2. It will use state-of-the-art virology, molecular biology, and immunology to answer questions related to behavior and disease progression in infected individuals.

3. It will integrate virologic and immunologic analyses of HIV-1 in the female genital tract into the scientific fabric of the study. Study of the female genital tract is important because it may serve as a major reservoir of HIV-1 and potential site for viral transmission, either sexual or vertical.

To ensure that the WIHS-NIDA study addresses the issues under investigation directly and uses the most recently developed, relevant scientific methods, virus and immune response in the CVL will be studied. Investigation of the CVL as part of the WIHS-NIDA study is important for a number of reasons. First, study of virus and immune response in the CVL will permit direct investigation of one of our major hypotheses: that HIV infection in women infected mucosally differs from that of women infected parenterally. It is possible that women who are infected mucosally and whose initial site of HIV-1 infection is the genital tract have a major reservoir of virus in the genital tract that differs in both quantity and quality from those infected parenterally. Sexual transmission of HIV-1 is the major route of infection worldwide and accounts for more than half of the transmission to women in the U.S. Investigation of HIV-1 infection in the genital tract is necessary to understand HIV-1 pathogenesis, vaccine design, and heterosexual and mother-to-child transmission, yet relatively little research has focused on this area to date.

At this time, this NIDA cohort is well suited for an integrated study of the virology and immunology of HIV-1 infection in the female genital tract and blood. In addition, all WIHS participants undergo a CVL for study at each visit; all of the studies we propose can be performed on a portion of the WIHS CVL specimen.

Recent studies by WIHS investigators and others have demonstrated the capability of quantitating and characterizing HIV-1, particularly HIV-1 RNA, in the CVL. Furthermore, investigation of HIV-1 infection and mucosal immune response in the genital tract focuses on an area that was judged a high priority by the external WIHS review: gender-specific issues related to HIV disease progression.

D. PARTICIPANT ELIGIBILITY AND ENROLLMENT

The scientific design and all matters relating to patient participation were discussed and approved by the National Community Advisory Board (NCAB). The NCAB prepared a letter to the WIHS participants introducing the study and encouraging enrollment.

To initiate the WIHS-NIDA study, we selected 302 HIV-1-infected and 79 uninfected women enrolled in the WIHS who represent three major patterns of drug use (see Appendix A for a list of those eligible to participate in the NIDA Immunology/Virology Substudy). Identification of patterns of drug use was based on self-reported information in the WIHS database for visits 1 and 2.

- Group I (no drug use) consists of women in the WIHS who reported no history of either injecting drug use (IDU) or non-injecting use of crack, cocaine, or heroin (CCH) at any time in their past.
- Group II (recent CCH use) consists of women who reported non-injecting use of CCH in the six months preceding visit 1, visit 2, or both visits but gave no history of any IDU.
- Group III (recent IDU), by contrast, consists of those who did report injecting drug use in the six months preceding visit 1, visit 2, or both visits 1 and 2.

Groups II and III were defined with the goal of identifying sizable groups of women with a history of recent drug use and the understanding that drug use is frequently intermittent. For analysis of this study, a woman whose only risk for HIV-1 is heterosexual transmission is presumed to have been infected through a mucosal route while a woman who injects drugs and is sexually active may have been infected either parenterally or mucosally.

Women were stratified according to CD4 count for basic classification of disease stage. As one method of addressing the issue of antigenic and immunologic activation, women were stratified according to lifetime number of male sexual partners as well. Both injecting and non-injecting drug use has been associated with multiple sexual partners and consequent sexually transmitted diseases which may lead to immune activation. To assess the possible role of the number of sexual partners on both clinical disease progression and viral and immunologic factors, women were stratified in all three drug use categories according to number of male partners.

Table 1A presents the WIHS-NIDA cohort categorized by drug use group, CD4 count, and number of lifetime male sexual partners. All of the HIV-1-infected women in Groups II (CCH) and III (IDU) were chosen for investigation in order to maximize the numbers of drug-using women; previous studies have indicated that individuals with a recent history of drug use may be more likely to be lost to follow up.

Table 1B shows the HIV-1-uninfected women who were selected as controls for immunologic studies. These women were also stratified according to the three major drug groups and number of male sexual partners; in addition they were chosen to parallel, when feasible, the drug groups, number of sexual partners, age, and geographic site of the infected women.

E. OVERVIEW OF VISIT

The visit components of the NIDA Immunology/Virology Substudy for baseline and follow-up visits are as follows:

1. BASELINE

    a. Interview

        Participant Notification Form (NVNOTI)

NIDA I/V Enrollment Interview (NV01)

b. Specimen Collection

i. Blood Collection (NV03)
- 1 additional EDTA (8 ml) tube
- 2 additional Heparinized (8 ml each) tube (intensive analysis subset only)

ii. Urine Collection – 30 ml urine (NV03)

iii. CVL – for all odd visits, a total of 4.2 ml of CVL will be taken from the total amount collected from the WIHS core visit

2. FOLLOW-UP

a. Interview

There is no interview portion for NIDA I/V follow-up visits. Data on antiretroviral drug use will instead be collected on WIHS core forms F22, DG1 and DG2.

b. Specimen Collection

i. Blood Collection (NV03)
- 1 additional EDTA (8 ml) tube
- 2 additional Heparinized (8 ml each) tube (intensive analysis subset only)

ii. Urine Collection – 30 ml urine (NV03)

iii. CVL – for all odd visits, a total of 4.2 ml of CVL will be taken from the total amount collected from the WIHS core visit

More information regarding the individual components of the visit follow in the next section.

Collection of data for prospective study will end as of September 30, 2001.

F. VISIT PROCEDURES

1. INTERVIEW

A limited number of extra questions will be asked of each participating subject when she joins the NIDA study. These questions will be added to the standard WIHS interview. They are aimed at obtaining more detailed information about the patients' history of drug use, both street drugs and antiretroviral drugs. Antiretroviral drug use, which used to be collected on form NV02, will now instead be collected on WIHS core forms F22, DG1 and DG2. There will therefore be no interview portion of the visit for all follow-up NIDA I/V visits.

2. LABORATORY STUDIES

All women in the WIHS currently have plasma HIV-1 RNA loads performed at each visit as part of the core protocol of the WIHS. As part of this substudy, the WIHS-NIDA cohort will have viral loads performed on their CVL's annually in addition, as well as tests of immune function and activation. The virologic and immunologic studies will be performed serially on all of the infected participants, with immunologic studies alone performed on the uninfected controls.

Up to four different specimens are collected and processed for the NIDA Immunology/Virology Substudy. These specimens obtained specifically for NIDA are to be collected during the WIHS Medical Exam **following** the specimens collected for the core WIHS study. All specimens collected for NIDA are sent to a laboratory for processing, so none of the specimens require immediate processing at the WIHS site unless the NIDA lab is also a WIHS lab.

The four specimens that are collected for NIDA are as follows:

- 1 EDTA tube collected on all NIDA participants at every visit

- 2 Heparinized tubes collected only on subset of 56 at every visit (see Section G for more information on subset of 56)
- 4.2 ml of CVL taken from core CVL collection on all NIDA participants at every odd visit (annually)
- 30 ml of urine collected at every visit on all NIDA participants who provide consent (urine toxicology is an optional test)

See Appendix B: Flow Chart of NIDA Specimens for further information.

3. BLOOD COLLECTION, PROCESSING AND SHIPMENT

One additional EDTA tube (8 ml) will be required of all participants at each visit. A total of three additional tubes (1 EDTA and 2 heparinized; 24 ml) will be required of the 56 individuals selected for intensive study at each visit.

a. EDTA tube

i. Collection and shipment

One EDTA tube is collected per NIDA participant at each visit. A total of 8 ml of blood should be collected in each EDTA tube. Once collected, be sure that the anticoagulant and the blood are mixed prior to shipment. EDTA tubes are then shipped overnight at room temperature to Jim Bremer's laboratory at Rush Presbyterian St. Luke's Medical Center in Chicago, IL.

ii. Processing

Once the EDTA tubes are received at Dr. Bremer's lab, a total of 1 ml will be removed immediately and sent to Dr. Alan Landay's lab in the same building for immunologic studies. The remaining 7 ml will then be processed at Dr. Bremer's lab as follows:

a) Subset of 56

Separate EDTA-treated blood into plasma and cells by Ficoll Hypaque. Once plasma is split into 0.5 ml aliquots and cells into dry pellets containing 500,000 cells each, ship as follows:

To Barbara Weiser:	2 ml plasma (4 x 0.5 ml) and 2 million cells (4 pellets)
To Jim Bremer:	2 ml plasma (4 x 0.5 ml) and 2 million cells (4 pellets)
To WIHS-NIDA repository:	Remaining plasma (0.5 ml aliquots) and cells (500,000 cells/pellet)

NOTE: The WIHS-NIDA repository is located at Jim Bremer's lab in Chicago, IL

b) Remainder of NIDA cohort

Separate EDTA-treated blood via centrifugation at 400 x g for 15–20 minutes. After plasma and cells have been separated, split plasma into 0.5 ml aliquots. Reconstitute cell pellet with PBS and lyse RBCs using Roche cell prep method. Split WBC into 0.5 ml aliquots. Ship both plasma and cells as follows:

To Alan Landay:	0.5 ml plasma (1 x 0.5 ml)
To WIHS-NIDA repository:	Remaining plasma (0.5 ml aliquots) and WBC (0.5 ml aliquots)

NOTE: The WIHS-NIDA repository is located at Jim Bremer's lab in Chicago, IL

b. Heparinized tubes

i. Collection and shipment

A total of two heparinized tubes are collected at each visit only for those 56 participants who have been selected for intensive study in NIDA. A total of 10 ml is to be collected in each heparinized tube for a total of 20 ml. Once collected, be sure that the anticoagulant and the blood are mixed prior to shipment. Both Heparinized tubes are then shipped overnight at room temperature to Jim Bremer's laboratory, *unless the participant is an LA participant.* If the participant is from LA, then one tube is sent to Andrea Kovacs' laboratory and the other is sent to Jim Bremer's laboratory.

ii. Processing

Once both Heparinized tubes are received at Dr. Bremer's laboratory (only one tube will be received for all LA participants), blood will be separated by Ficoll Hypaque. Once separated into plasma and viable cells, split plasma into 0.5 ml aliquots and viable cells into aliquots of three million cells. One aliquot of three million cells will be used for cultivation by either Andrea Kovacs (LA participants) or Jim Bremer (all other sites). Ship remaining plasma and cell aliquots as follows:

To Alan Landay:	4 ml plasma (8 x 0.5 ml) and 3 million viable cells (1 aliquot)
To WIHS-NIDA repository:	Remaining plasma (0.5 ml aliquots) and viable cells (aliquots of 3 million cells)

NOTE: The WIHS-NIDA repository is located at Jim Bremer's lab in Chicago, IL

4. CERVICOVAGINAL LAVAGE COLLECTION, PROCESSING AND SHIPMENT

CVL samples (4.2 ml) once yearly on all of the WIHS NIDA participants will be studied. The timing of the CVL samples of these participants will be carefully coordinated with the needs of other WIHS studies. HIV-1 RNA load will be determined on all specimens. Samples from the 56 women chosen for intensive study will also be the subject of detailed virologic, molecular, and immunologic studies.

a. Collection and shipment

A total of 4.2 ml will be taken from the total amount of CVL collected for the core study at <u>every odd visit</u>. The 4.2 ml CVL will be shipped as follows:

Bronx/Brooklyn:	Barbara Weiser
DC/Chicago:	Jim Bremer
LA:	Andrea Kovacs

NOTE: The WIHS sites are to process the remaining 4.8 to 5.8 ml of CVL according to the WIHS Core CVL Protocol being followed at their particular site.

b. Processing

Upon receipt of the 4.2 ml of CVL, all three NIDA laboratories will perform HIV1-RNA quantification on 1.0 ml of CVL using the NucliSens assay. The remaining 3.2 ml of CVL will be aliquoted and shipped as follows:

i. Subset of 56

CVL will be split into 4 x 1.0 ml aliquots. Each NIDA lab (Dr. Weiser, Dr. Bremer, and Dr. Kovacs) will keep 1 ml. The remaining 3 ml (3 x 1.0 ml) will be sent to the each of the other NIDA labs as follows:

Dr. Weiser: Keep 1 ml, ship 1 ml aliquots to Dr. Bremer, Dr. Kovacs, and Dr. Landay

Dr. Bremer: Keep 1 ml, ship 1 ml aliquots to Dr. Weiser, Dr. Kovacs, and Dr. Landay

Dr. Kovacs: Keep 1 ml, ship 1 ml aliquots to Dr. Weiser, Dr. Bremer, and Dr. Landay

ii. Remainder of NIDA cohort

CVL will be split into 8 x 0.5 ml aliquots and shipped to WIHS-NIDA repository.

5. URINE TOXICOLOGY COLLECTION, PROCESSING AND SHIPMENT

Because WIHS data regarding drug use and consequent selection of women for this study are based upon self-report, it is important to perform urine toxicology as part of the WIHS-NIDA I/V study. Toxicology will be requested but not mandated of the participants. The purpose of the tests will be to verify the history of drug use reported by the women and to obtain a very rough measure of frequency of active drug use.

Several procedures will be used to guarantee participants' rights and confidentiality including: 1) patient consent, 2) certificate of confidentiality from Department of Health and Human Services, and 3) assurance of confidentiality of study records. Participants will be assured that they will be able to participate in the substudy without consenting to urine toxicology, but that the assay is highly desirable and will increase our information. The National Community Advisory Board letter to participants explains the rationale for drug testing and measures to ensure confidentiality.

For WIHS visits already completed, 1.5 ml of urine in the repository will be examined. For future visits, a total of 30 ml of urine will be collected, upon consent, at every visit. The central laboratory that will be testing the urine is Northwest Toxicology Inc. (NWT Inc.) in Salt Lake City, UT. NWT Inc. will provide each site with collection and shipping kits.

Urine can be stored locally for up to a week at either room temperature or in a refrigerator prior to shipment to NWT Inc. Urine can either be shipped daily or batched weekly and is shipped via U.S. Mail.

G. INTENSIVE ANALYSES ON A SUBSET OF PARTICIPANTS

For more focused study, intensive molecular, virologic, and immunologic analyses will be performed on a subset of 56 of the infected participants in the cohort (see Appendix A for a list of participants in the subset). These studies will include analyses of HIV-1 RNA sequence diversity, antiviral resistance, viral tropism, and detailed studies of cell-mediated immunity, cytokines, and chemokines. This subset, described in Table 2, has been designed to represent the same broad spectrum of drug use patterns and number of sexual partners as the larger cohort. To derive maximal scientific information from the intensive investigations of these participants, they have been selected based on plasma HIV-1 RNA load rather than CD4 count. Plasma HIV-1 RNA load measured on visit 1 was used. "High load" participants represent the women in each category in Table 2 who had the highest viral loads. "Low load" participants represent two women in each category with HIV-1 RNA levels in the low but detectable range (approximately 2,000-5,000 copies of HIV-1 RNA/ml) and two women with viral loads <4000 (undetectable using current tests).

Results of the various viral and immunologic assays will be analyzed and correlated with each other. In addition, data will be analyzed to study virology, immunology, and clinical HIV-1 disease as they relate to route of infection, number of sexual partners, and antigenic and immune stimulation.

H. ADDRESSES OF WIHS-NIDA LABORATORIES

1. James Bremer, PhD (also serves as WIHS-NIDA repository)

Ship to: Cheryl Jennings
Rush Presbyterian St. Lukes Medical Center
Retrovirology Lab, 1181 Jelke
1750 West Harrison Street
Chicago, IL 60612

Phone: (312) 942-3446
FAX: (312) 942-6787

2. Andrea Kovacs, MD

Ship to:

Patricia Yepez
USC School of Medicine
GLB Room 1G8
1801 East Marengo St.
Los Angeles, CA 90033

Phone: (213) 226-4161
FAX: (213) 226-4168

3. Alan Landay, PhD

Ship to:

Mary Ann Czerniewski
Rush Medical Center
Dept. of Immunology/Microbiology
1750 West Harrison St., 1537 Jelke
Chicago, IL 60612

Phone: (312) 942-5801
FAX: (312) 942-2808

4. Barbara Weiser, MD

Ship to:

Barbara Weiser
Wadsworth Center
NYS Dept. of Health
David Axelrod Institute
New Scotland Avenue
Albany, NY 12208

Phone: (518) 473-3546
FAX: (518) 473-4110

5. Urine Toxicology laboratory

Ship to:

NWT Inc.
1141 East, 3900 South
Suite A110
Salt Lake City, UT 84124

Phone: (801) 268-2431
FAX: (801) 263-3605

TABLE 1:
NUMBERS OF WOMEN CATEGORIZED BY DRUG USE GROUP, CD4 COUNT, AND NUMBER OF LIFETIME MALE SEXUAL PARTNERS

(These numbers are based on the initial projected participation of six sites and have since been reduced. These participants may still be included in analysis.)

A. HIV-INFECTED WOMEN

Drug Group I		**Numbers of Lifetime Male Partners**			
Never injected, never used CCH*		**1-3**	**4-10**	**>10**	**Totals**
CD4 count	0-199	10	10	10	30
	200-499	20	20	20	60
	≥500	10	10	10	30
Group I subtotal		**40**	**40**	**40**	**120**
Drug Group II					
Recent CCH, never IDU					
CD4 count	0-199	1	15	21	37
	200-499	3	14	35	52
	≥500	2	4	23	29
Group II subtotal		**6**	**33**	**79**	**118**
Drug Group III					
Recent IDU					
CD4 count	0-199	4	18	21	43
	200-499	6	18	38	62
	≥500	4	12	29	45
Group III subtotal		**14**	**48**	**88**	**150**
Total Groups I, II, and III					**388**

B. HIV-UNINFECTED CONTROLS

	Numbers of Lifetime Male Partners			
	1-3	**4-10**	**>10**	**Totals**
Drug Group I **Never injected never used CCH**	6	11	17	34
Drug Group II **Recent CCH, never IDU**	3	14	17	34
Drug Group III **Recent IDU**	4	13	17	34
Total Groups I, II, and III	**13**	**48**	**51**	**102**

*CCH; Non-injecting use of crack, cocaine, or heroin

TABLE 2:
SUBSET OF INFECTED WIHS-NIDA COHORT SELECTED FOR INTENSIVE LABORATORY STUDIES

Drug Group I, Never Injected, Never Used CCH*

	Numbers of Lifetime Male Partners			
	1-3	**4-10**	**>10**	**Total**
Plasma HIV-1 RNA Load				
High	4	4	4	12
Low	4	4	4	12
Group 1 Subtotal	**8**	**8**	**8**	**24**

Drug Group II, Recent CCH, Never IDU

	Numbers of Lifetime Male Partners		
	1-10	**>10**	**Total**
High	4	4	8
Low	4	4	8
Group 2 Subtotal	**8**	**8**	**16**

Drug Group III, Recent IDU

	Numbers of Lifetime Male Partners		
	1-10	**>10**	**Total**
High	4	4	8
Low	4	4	8
Group 3 Subtotal	**8**	**8**	**16**

Groups I, II, and III Total **56**

***CCH, Non-injecting use of crack, cocaine, or heroin**

APPENDIX A: LIST OF THOSE ELIGIBLE FOR NIDA IMMUNOLOGY/VIROLOGY

List of those eligible for NIDA Immunology/Virology from the Bronx

ID	SUBSET	ID	SUBSET
10100153		10102525	
10100329		10102537	
10100406	Yes	10102563	
10100420		10102614	
10100583		10102664	Yes
10100595		10102676	Yes
10100696		10102703	
10100761		10102753	Yes
10100848	Yes	10102765	
10100850		10102828	
10100874		10102854	
10100951		10102866	
10101028		10102931	Yes
10101042		10102955	
10101054		10102993	
10101066		10103060	
10101167		10103111	Yes
10101181		10103147	
10101422		10205218	
10101496		10205369	
10101523		10205458	Yes
10101547		10205460	
10101636		10205496	
10101650		10205624	Yes
10101662		10205636	
10101698		10205698	
10101713		10205890	
10101725		10205953	
10101927		10206018	
10101989	Yes	10206032	
10102195		10206044	
10102222		10206094	
10102311		10206145	
10102424		10206412	Yes
10102436		10310069	
		10310348	
		10310398	

List of those eligible for NIDA Immunology/Virology from Brooklyn

ID	SUBSET	ID	SUBSET
20100193		20102159	
20100294		20102224	
20100371		20102248	
20100383		20102351	
20100446		20102452	
20100484		20102464	
20100559	Yes	20204016	
20100600		20204155	
20100612		20204193	
20100648		20204256	
20100674	Yes	20204369	
20100864		20204408	Yes
20101145		20204458	
20101183	Yes	20204460	
20101208		20204509	
20101234		20204511	
20101260	Yes	20204523	
20101323		20204612	
20101335	Yes	20204650	
20101361		20204751	
20101450		20204787	
20101486	Yes	20204864	Yes
20101513		20204876	
20101599		20204927	
20101727	Yes	20204941	
20101804		20205068	
20101878		20205094	
20101905		20205107	
20101931		20205121	
20101955		20205171	
20102046		20205284	
20102058		20205335	
20102096	Yes	20205361	
		20205397	

List of those eligible for NIDA Immunology/Virology from DC

ID	SUBSET	ID	SUBSET
30100145		30306393	
30100347		30306420	
30100385		30306468	Yes
30100424		30409024	
30100474		30409036	
30100537		30409125	
30100602		30409163	
30100652		30409175	
30100676		30409187	
30100690	Yes	30409226	
30100804		30409238	
30100816		30409252	
30100828		30409288	
30100830		30409391	
30100878		30409428	
30100967		30409480	
30203030		30409505	
30203218		30409531	
30203270		30409579	
30203395		30409632	
30203472		30409668	
30203585		30409757	
30203650		30409808	
30203749		30512011	
30203751	Yes	30512061	
30306014		30512097	
30306064		30512112	
30306141	Yes	30512150	
30306153	Yes	30512287	
30306191	Yes	30512390	
30306305		30512631	
		30512720	

List of those eligible for NIDA Immunology/Virology from LA

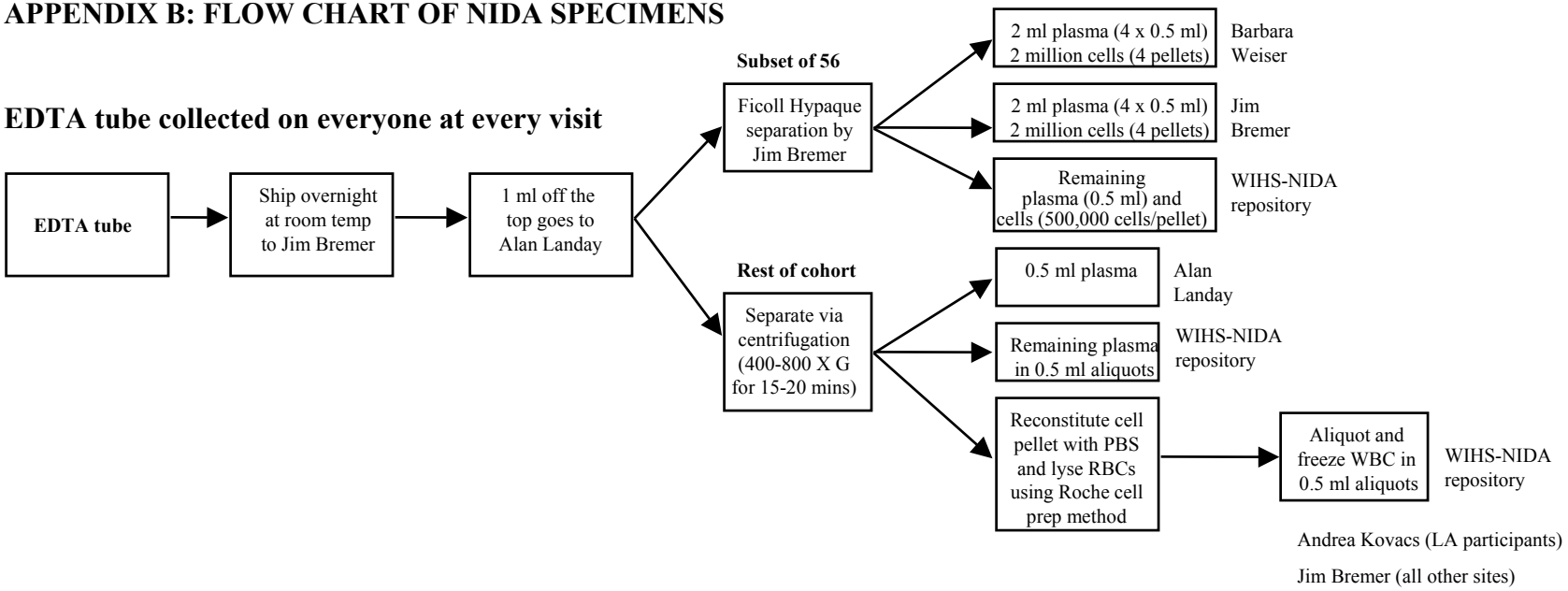
ID	SUBSET	ID	SUBSET
40100010		40718122	
40100034		40718209	
40100197		40718300	
40100224		40718312	
40100236	Yes	40718348	
40100337		40718362	
40100351		40718398	
40100363		40718413	
40100464		40718463	
40100488		40718588	
40100490	Yes	40924054	
40100577		40924080	Yes
40203018	Yes	40924143	
40203032		40924181	
40203121		40924294	Yes
40203171		40924371	Yes
40203208	Yes	40924410	Yes
40203272		41027116	
40203284		41027178	Yes
40203323	Yes	41027281	
40203513		41027332	
40203525		41027419	Yes
40203549		41027471	
40203575		41027508	
40203602		41027522	
40203640		41027546	
40203688	Yes	41027584	Yes
40203703		41027623	
40203741		41027697	Yes
40203789		41027724	
40203828		41027736	
40203842		41027798	
40203905		41027801	
40306066		41027813	Yes
40306167		41027899	
40306193		41027938	
40409064	Yes	41028081	
40409090		41028170	
40409127		41028233	Yes
40409177		41028295	
40409191		41028322	
40512102		41028384	
40512114		41028435	
40615059		41130026	
40615061		41130064	
40615225		41130115	
40615251		41130139	
40718021		41130177	
40718083		41130280	
		41130292	
		41130317	

List of those eligible for NIDA Immunology/Virology from Chicago

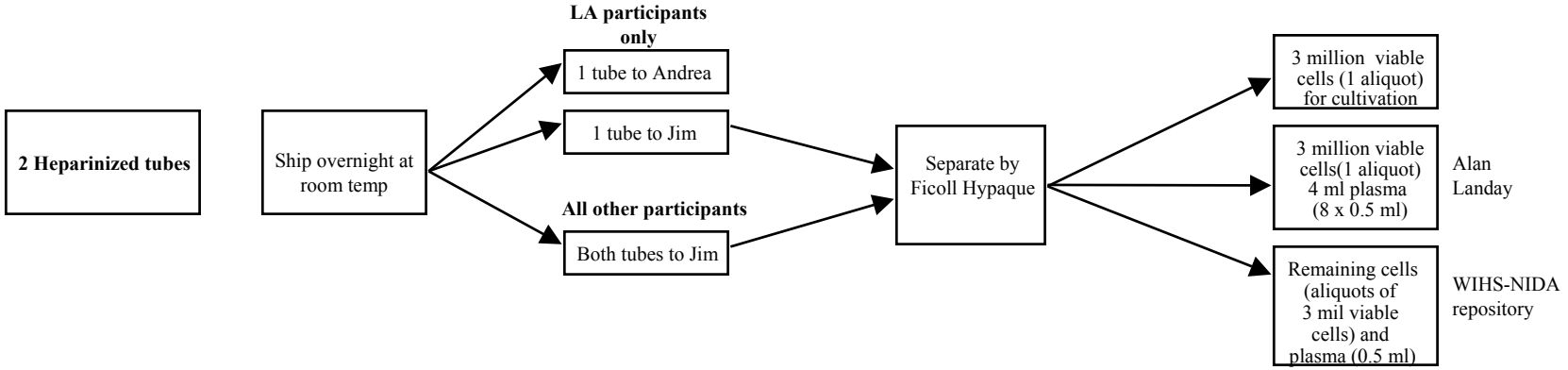
ID	SUBSET	ID	SUBSET
60100026		60202325	
60100076		60202375	
60100088		60202399	
60100090		60202440	Yes
60100139		60202464	
60100153		60202476	
60100216	Yes	60202503	
60100230		60202515	
60100317		60202591	
60100393		60304042	
60100406		60304155	
60100507		60304181	
60100569		60304244	
60100696	Yes	60304282	
60100747		60304319	Yes
60100785		60304357	
60100812	Yes	60304383	Yes
60100824		60304422	Yes
60100850		60406113	
60100925		60406137	
60101004		60406175	
60101028	Yes	60406187	
60101080		60406202	
60101167		60406252	
60101181		60406288	Yes
60101206		60406339	
60101220		60406341	
60101383		60406353	
60101395		60406428	
60101408		60406442	
60101446		60406478	
60101472		60406529	
60101509		60406543	
60202060		60406581	
60202197		60406620	
60202248		60406632	
60202262		60406656	Yes
60202298	Yes	60406721	
60202301	Yes	60406795	
		60406822	

APPENDIX B: FLOW CHART OF NIDA SPECIMENS

EDTA tube collected on everyone at every visit

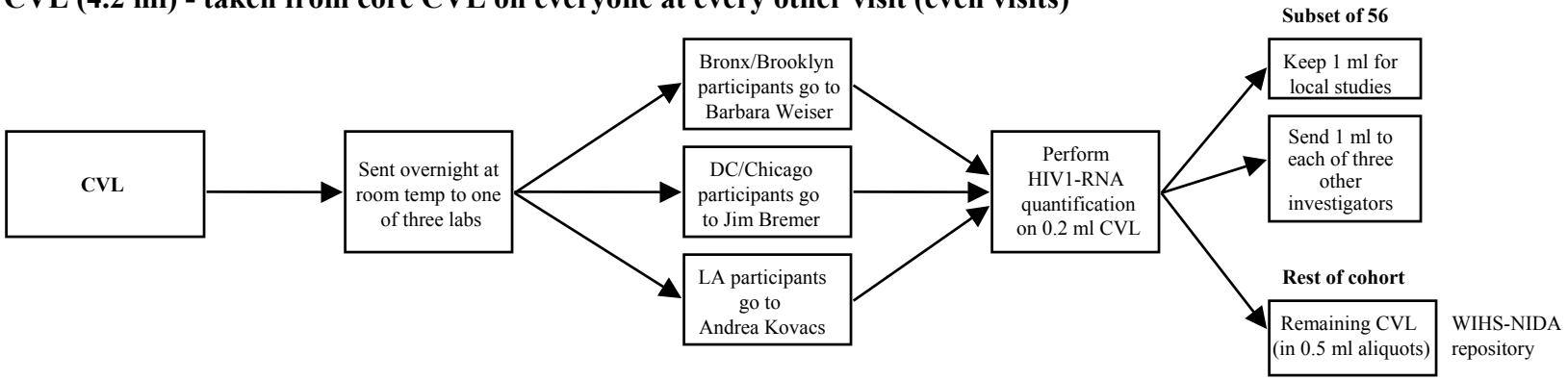


Heparinized tubes - two collected on subset of 56 at every visit



APPENDIX B: FLOW CHART OF NIDA SPECIMENS, CONT'D

CVL (4.2 ml) - taken from core CVL on everyone at every other visit (even visits)



Urine (30 ml) - collected at every visit separately from core WIHS protocol (optional)