Serial Plasma/PBMC Collections from Recently HIV-Infected Individuals

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INTRODUCTION

Serial plasma and cell samples from recently HIV-infected individuals, collected early in infection and at regular, frequent intervals over at least a year, are an unmet need for HIV research, for HIV incidence/prevalence studies, and for Precision Medicine research and interventions.

HIV seroconversion panels and the Fiebig staging algorithm (described in Table 1) provide an understanding of the laboratory profile of recent infection, and allow accurate estimates of infection dates based on a single sample collected during the first five months of infection.

Tests for HIV RNA among high-risk, HIV antibody negative individuals provide a means to detect very recent infections. SeraCare partnered with the San Francisco Department of Public Health (SFDPH) to recruit three recently-infected individuals for a one-year collection program

STUDY DESIGN/METHODS

A study protocol was approved by the SeraCare and SFDPH Institutional Review Boards based on the collection schedule described in Table 2.

As standard care, SFDPH pool-tests samples from HIV antibody negative STD clinic patients. From these results, SFDPH identified, recruited, and obtained consent from three HIV RNA positive/anti-HIV negative patients who will each provide 14 whole blood collections over 11 months, including four in the first two weeks, with monthly collections for ten months following.

Whole blood is shipped to SeraCare for processing into plasma and PBMCs. Aliquots (0.5 mL plasma, 10M cells/mL PBMCs) are available for research use as individual samples or as series. A Certificate of Analysis including clinical and demographic data, estimated infection date, and test results for HIV RNA and BED incidence tests will accompany the samples.

Table 1. Fiebig Stages in Early HIV Infection[1]

Fiebig Stage	Duration of each phase in days (CI)	Cumulative duration in days (CI)
Eclipse	10 (7,21)	10 (7,21)
I (vRNA +)	7 (5,10)	17 (13,28)
II (p24 Ag +)	5 (4,8)	22 (18, 34)
III (ELISA +)	3 (2,5)	25 (22,37)
IV (Western Blot ±)	6 (4,8)	31 (27,43)
V (Western Blot +, P31 -)	70 (40,122)	101 (71,154)
VI (Western Blot +, p31 +)	Open -ended	

RESULTS

Table 2. Collection Schedule

Time elapsed from recruitment date	# Collections	Volume/ collection	Total volume (8 weeks)	Total collections (cumulative)
Week 1 and 2	**2 each week**	**80 mL**	**400 mL (including month 1)**	**4**
Month 1	**1 collection**	**80 mL**		**5**
Months 2-10	**1 collection**	**80 mL**	**160 mL (including previous or next month)**	**14**

Table 2 (left) Description

The concept is to collect serial samples in sufficient volume to be useful over time, while remaining well within recognized volume limits for whole blood. Such samples may be useful to HIV early infection researchers, and to developers and evaluators of tests for HIV incidence.

Participants were provided compensation for each collection, and an incentive to complete the series. All three participants are expected to complete their series, two in January and one in February.

Table 4 (right) Description

Infection dates were estimated for each participant from his Fiebig stage[1,2] on the date of his HIV RNA positive/anti-HIV negative sample (eligibility date). All three participants were HIV antigen negative on their eligibility dates, according to a 4th gen HIV Ag/Ab test, and thus are estimated to be 17 days from infection on their eligibility dates. Samples from the first four bleed dates were tested for HIV RNA at SeraCare (see Table 5).

Table 4. Days from Estimated Infection Date for First Four Collections

Patient ID	Estimated infection date	Eligibility Test Date	ART Started	Bleed 1	Bleed 2	Bleed 3	Bleed 4
LSS102	0	17	44	39	43	45	48
LSS103	0	17	NA	43	47	50	55
LSS104	0	17	NA	24	26	30	36

Table 3. Test Results from Eligibility Date and Follow-up Appointment

	LSS102 Results		LSS103 Results		LSS104 Results	
Test	**Eligibility date**	**4 days later**	**Eligibility date**	**5 days later**	**Eligibility date**	**7 days later**
Rapid HIV-1	Neg	Neg	Neg	Neg	Neg	NT
HIV-1 RNA pool test	10,000+ c/mL	NT	10,000+ c/mL	NT	<10,000 c/mL	NT
HIV Ag/Ab Combo	Neg	Neg	Neg	POS	Neg	Neg
HIV-1 IFA	Neg	Neg	Neg	POS	Neg	Neg
HIV-1 Western blot	Neg	Neg	Neg	POS	Neg	Neg
HIV-1/HIV-2 rapid	Neg	Neg	Neg	NT	Neg	Neg
HIV-1 quant RNA	NT	1.4x10e5	NT	2.4x10e6	NT	5.1x10e2
Fiebig Stage	**I**	**I**	**I**	**V**	**I**	**I**

Table 3 (above) Description

Results from each participant's eligibility appointment (when the HIV RNA positive/anti-HIV negative sample was drawn), and from follow-up appointments, when the participant was counseled and offered an opportunity to join the study, are listed here. These are SFDPH results.

Table 5 (below) Description

The first four (early infection) collections from each participant were tested at SeraCare for quantitative HIV RNA, and results are listed in Table 5. Incidence test results from BED and LAg methods will be available for all collections when series are complete for each participant. Testing has been limited because available volume are small.

Table 5. HIV RNA Test Results from Early Infection Collections

LSS102		LSS103		LSS104	
Estimated Days Post Infection	**HIV Viral Load**	**Estimated Days Post Infection**	**HIV Viral Load**	**Estimated Days Post Infection**	**HIV Viral Load**
39	2.95E+04	43	2.08E+05	24	5.04E+02
42	3.51E+04	47	1.48E+05	26	4.98E+02
45	3.64E+04	50	1.09E+05	30	1.88E+04
48	1.78E+03	55	4.78E+04	36	4.97E+04

CONCLUSIONS

- **For the first time, serial plasma and PBMC samples with informed consent, collected over a year from individuals recruited at Fiebig stage I, are available for research on early HIV infection, for HIV incidence/prevalence studies, or for other HIV research.**
- **Single samples or series are available, each provided with demographic and clinical information and quantitative HIV RNA results. Incidence test results will be added when collections are complete.**
- **Test results are limited due to the small overall volume available. Published or contributed test results will be added to the appropriate Certificates of Analysis.**
- **Plasma and PBMCs from four bleeds collected over two weeks within the first 60 days following infection may reveal new information on the evolution of viral markers or the immune response during this period.**
- **Collections are continuing, with an anticipated completion in January and February 2013 of 14 collections spanning about 11.5 months from each of three participants.**

REFERENCES

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