Title:	Collection and Processing of Semen		
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1 Purpose

The purpose of this SOP is to provide a standardized method for the collection and processing of semen samples and their components (seminal plasma and seminal cells) from participants enrolled in ACTG or IMPAACT clinical trials.

2 Scope

Users of the ACTG/IMPAACT Lab Manual.

3 Background

Semen is a major vehicle for the sexual transmission of HIV-1. The ability to isolate infectious HIV from the semen and to quantitate viral burden in the form of cell-free or cell-associated HIV-1 RNA in semen are important for epidemiologic and public health aspects of the epidemic. Earlier studies used viral culture to detect HIV in semen. Cell associated culturable virus recovery rates ranged from 8 to 55% [11.1, 11.12, 11.18, 11.20-23]. Much lower recovery rates (3-15%) were reported by these investigators for cell-free seminal-plasma viral load (>3.5 to 4 log), and an AIDS diagnosis was more apt to have positive seminal cell HIV cultures.

The application of quantitative HIV RNA and DNA assays demonstrated over twenty years ago that 60-75% of men shed HIV RNA in the seminal plasma and that 65-80% have detectable HIV DNA in seminal cell pellets [11.6, 11.8, 11.10-11, 11.14, 11.16, 11.21, 11.23-24]. Cross-sectional studies using commercially available RNA kits concluded that seminal plasma RNA levels are significantly correlated both with blood plasma RNA levels [11.10, 11.23], and the recovery of infectious virus from seminal cells [11.6, 11.23] but not with CD4 cell count [11.6, 11.10, 11.23], stage of disease [11.6, 11.23] or antiviral therapy [11.6, 11.10, 11.23]. Multiple studies since then have confirmed these early findings [11.4, 11.7, 11.15, 11.17].

A recent prospective study showed that higher seminal HIV-1 RNA concentrations are associated with greater risk of heterosexual HIV-1 transmission, and this effect was independent of plasma HIV-1 concentrations [11.3]. These data suggest that HIV-1 RNA in genital secretions could be used as a marker of HIV-1 sexual transmission risk. Therefore, knowledge of the effect of receptive topical microbicides and/or antiretrovirals on reduction of viral load in semen is necessary for the evaluation of compounds that may be useful in prevention of transmission and on establishing the clearance of HIV-1 from the male genital tract following effective “HIV-cure” interventions [11.13]. It has been demonstrated in longitudinal studies that the amount of HIV RNA in seminal plasma increases with time in individuals who progress to AIDS [11.9] and decreases with effective antiviral therapy [11.2, 11.9-10], but may remain detectable in persons who are virally suppressed in the plasma [11.19]. Antibiotic treatment of pathogens causing urethritis, especially gonorrhea, can also reduce seminal plasma viral load [11.5]. In addition, since the viral burden in genital secretions may serve as a reservoir in patients who have had their virus seemingly eliminated from the peripheral blood, the quantitation of virus in semen has become a major focus of clinical trials. The following procedures can be used for conducting studies involving semen.

4 Budgetary Considerations

4.1 Specialized supplies

4.2 Room or facility charges

4.3 Participant reimbursement

4.4 Sample analyses

4.5 Shipping Costs

5 Authority and Responsibility

5.1 The Network Laboratory Directors (or his/her designee) have the authority to establish, review and update this procedure.

5.2 The ACTG/IMPAACT Laboratory Technologist Committee (LTC) is responsible for the maintenance and control of SOP documentation.

5.3 The Laboratory Director is responsible for the implementation of this LTC SOP or laboratory-specific SOP and for ensuring that all appropriate personnel are trained. A laboratory SOP must:

5.3.1 Include, without procedural modification, the portions of the current version of the LTC SOP that are used within the network site-affiliated laboratory.

5.3.2 Reference the current version of the LTC SOP.

5.4 All laboratory technicians are responsible for reading and understanding this SOP prior to performing the procedures described.

5.5 The site PI and designees are responsible for understanding and adhering to the participant preparation and specimen collection components.

6 Eligibility Requirements

6.1 Inclusion Criteria:

6.1.1 Able to give informed consent.

6.1.2 Agree to refrain from sexual activity for at least 48 hours prior to donation.

6.1.3 Protocol-specific inclusion criteria.

6.2 Exclusion Criteria:

6.2.1 Significant genital symptoms which, in the opinion of the study investigator, represent a contraindication to genital sampling.

6.2.2 Protocol-specific exclusion criteria.

7 Equipment, Consumables and PPE

7.1 Individually wrapped antiseptic towelettes

7.2 Sterile urine collection cup or other collection container specified in the protocol MOPs and/or LPC

8 Reagents and Reagent Preparation

8.1 PBS, Ca and Mg-free

8.2 RPMI 1640

8.3 Hanks Balanced Salt Solution (HBSS)

8.4 Ficoll-hypaque or LSM

8.5 Trypan blue

9 Procedure

9.1 Participant Preparation and Specimen Collection

9.1.1 Instruct participant to refrain from sexual activity for at least 48 hours prior to donation.

9.1.2 Instruct participant to wash his hands and penis and then use an antiseptic towelette to wipe the head of the penis including the opening. If the participant is uncircumcised, the foreskin should be pulled back before cleaning the head and opening.

9.1.3 Instruct participant to masturbate and collect the specimen in a sterile container, for example a sterile urine collection container. Note: for some PK samples, a specific collection container may be required.

9.1.4 Participant or clinician must record the time that the specimen was produced on paperwork accompanying the specimen.

9.1.5 Provide details regarding any necessary post-sampling procedures for the participant.

9.2 Specimen Transport

9.2.1 Place the container in a zip-lock bag with absorbent and then in an appropriate biohazard transport carrier. The mode of transport will determine the type of carrier required. For example, specimens transported by cab must comply with DOT regulations for infectious substances. Package the sample to keep the specimen container upright.

9.2.2 Transport the specimen rapidly at room temperature to the clinic or directly to the laboratory. The laboratory should receive the specimen as soon as possible, preferably within 2 hours.

9.3 Specimen Processing

Note: Subsequent procedures should be performed in a class 2 biosafety laminar flow hood using sterile technique and adhering to CDC/NIH standards (including the use of gloves, eye protection and laboratory coats).

9.3.1 Allow liquefaction of semen to occur. At room temperature, this typically occurs within 20-45 minutes of specimen collection.

9.3.2 Whole semen:

9.3.2.1 Aliquot as instructed by protocol documents into cryovials.

9.3.2.2 Freeze at -70°C or colder. (LDMS code = SEM/NON/SEM)

9.3.3 Fractionated semen:

9.3.3.1 Transfer the sample to a conical centrifuge tube using a pipette. Measure and record the volume of semen, noting anything unusual about the sample (i.e. contaminated with RBCs, very viscous, etc.)

9.3.3.2 Centrifuge semen at 600-800 x g for 10 minutes.

9.3.3.3 Remove supernatant (seminal plasma), divide in 0.5 – 1.0 mL aliquots and freeze at -70°C or colder. Seminal plasma can be used for measurement of HIV RNA, PK, antibodies, cytokines, etc. (LDMS code = SEM/NON/FLD)

9.3.3.4 Wash cells by re-suspending cells in the pellet from above in 5.0mL RPMI, HBSS or PBS.

9.3.3.5 Centrifuge at 400-600 x g for 10 minutes.

9.3.3.6 *Note:* At this point some investigators remove spermatozoa from the cellular fraction using a standard ficoll-hypaque gradient. For example, this may be important for some studies in which HIV proviral DNA is investigated. DNA found in sperm may interfere with the analysis of HIV DNA. If the presence of spermatozoa proves to be a problem, perform the following:

- Layer the seminal cells onto 4mL of ficoll-hypaque.
- Centrifuge at 600 x g for 20 minutes.
- Carefully remove the non-spermatozoal cells found at the interface and resuspend in 10mL HBSS or PBS.
- Centrifuge at 400-600 x g for 10 minutes.
- If sperm cells do not interfere with anticipated assays, this ficoll separation does not need to be performed.

9.3.3.7 Repeat the wash steps (9.3.3.4 and 9.3.3.5) once and resuspend in 3mL RPMI, HBSS, PBS, or other reagent if indicated in the protocol LPC or MOP.

9.3.3.8 If required for the protocol, determine the viability of the cells using trypan blue dye exclusion method. An accurate cell count of the white blood cells will be difficult as they cannot be readily distinguished from immature germ cells. The number of cells will vary with each specimen. Record this number such that it may be retrieved if needed.

9.3.3.9 Centrifuge the remaining primary seminal cells for 10 minutes at 600-800 x g.

9.3.3.10 Remove the supernatant and then resuspend the remaining seminal cells in 2mL cryoprotective medium (DMSO containing freezing medium).

9.3.3.11 Divide into two 1.0mL aliquots and freeze following the procedure found in the Cross Network PBMC Cryopreservation SOP.

9.3.3.12 Long-term storage should be in gas phase LN2. Store up to 4 weeks at -80°C and ship to the repository with the cryopreserved PBMCs for long

term LN2 storage unless instructed differently by the protocol LPC. (LDMS code = SEM/NON/CLN/DMS)

10 Forms

A sample informed consent document for participants is attached as Appendix A.

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Appendix A: Sample Informed Consent

(Pertinent sections may be used in developing the Protocol Informed Consent documents)

_____MEDICAL CENTER

CONSENT FORM FOR HIV POSITIVE PARTICIPANTS

Collection of Specimens from Persons

Research Site personnel Contact information:

Name	Title	Phone #
_____	Professor, Medicine	(###) ###-####
_____	Associate Professor, Lab Medicine	(###) ###-####
_____	Acting Instructor, Medicine	(###) ###-####
_____	Research Nurse, Medicine	(###) ###-####
_____	Research Referral Nurse, Medicine	(###) ###-####
Emergency 24 hr. number	Page On-call AIDS Researcher	(###) ###-####

INVESTIGATOR'S STATEMENT

We are asking you to volunteer for a research study. The purpose of this consent form is to give you the information you will need to help you decide whether or not to be in the study. Please read the form carefully. You may ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. When all your questions have been answered, you can decide if you want to be in the study or not. This process is called “informed consent.”

PURPOSE OF THE STUDY

We are asking you to take part in this research study because you are infected with the human immunodeficiency virus (HIV), the virus that causes the acquired immune deficiency syndrome (AIDS). You may have no symptoms from the infection, mild symptoms, AIDS-related complex (ARC), or AIDS. The purpose of this study is to obtain biological samples to be used for laboratory studies of HIV.

PROCEDURES

If you consent to participate, we will ask you questions about selected aspects of our medical history. You are free not to answer any of these questions. If we determine that you are eligible to participate, you may have up to ###ml (about # tablespoons) of blood drawn from a vein in your arm one time. This visit for the study will take about 15 minutes. You may also be asked if you are willing to provide a semen sample. We may need to review your medical record for information about your HIV infection, such as medications you have taken and opportunistic infections you have had. We would like you to sign a separate Consent for Release of Information form for us to review your medical record.

RISK, STRESS, DISCOMFORT

Drawing blood may cause mild discomfort or a bruise. Questions about your health may be stressful. Risks of collection of semen samples include potential embarrassment.

ALTERNATIVES TO TAKING PART IN THIS STUDY

Taking part in this study is voluntary. You can stop at any time. Choosing to take part in this study, or choosing not to take part, will not affect your health care.

BENEFITS OF THE STUDY

You will not directly benefit from taking part in this research. However, we hope that the results of this research will help us in the future.

OTHER INFORMATION

Information about you is confidential. We will code the information you give us. The link between your name and the code will be kept in a secured location, separate from the study information. The link between your name and the study information will be kept indefinitely. If we publish the results of this study, we will not use your name.
Although we will make every effort to keep your information confidential, no system for protecting your confidentiality can be completely secure. It is possible that unauthorized persons might discover that you are in this study, or might obtain information about you. Government or university oversight offices sometimes reviews studies such as this one to make sure they are being done safely and legally. If a review of this study takes place, your records may be examined. The reviewers will protect your privacy. The study records will not be used to put you at legal risk of harm.

We will pay you $## for a blood draw of up to ##ml (about # tablespoons). For a semen collection we will pay you $##.

_____ _____

Investigator's Signature Date

_____

Investigator's Printed Name

PARTICIPANT'S STATEMENT

The study has been explained to me, and I voluntarily consent to participate. I have had an opportunity to ask questions. Future questions I may have about the research or research-related injuries will be answered by one of the investigators listed on page one. If I have questions about my rights as a research participant, I may call the _____ (XXX) XXX-XXXX. I give the investigators permission to review my medical records as described above. I will receive a copy of this consent form, and a copy may go into my medical record (if I give my permission).

_____ _____

Participant's Full Signature Date

_____

Participant's Printed Name

Copies to: Participant

Participant's Research File