

## **Section 10. Laboratory Procedures**

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### **10.1 Overview and General Guidance of Section 10**

This section contains instructions related to laboratory procedures required by HPTN 052.

Some laboratory procedures may be performed in study site clinics; others will be performed in study site local laboratories, and at the HPTN Network Laboratory (NL) in Baltimore, MD, USA. Table 10-1 lists the testing location for each test and kit requirements for each test. Protocol Section 5.0 and Appendix IA / IB specify the time points at which each test is to be performed.

In all settings, laboratory procedures will be performed according to study site standard operating procedures (SOPs) that have been approved by the HPTN NL.

Copies of all applicable package inserts and material safety data sheets should be accessible for reference in on site testing locations. Please contact the HPTN NL with any questions about these documents. Note: a package insert is not available for the S/P pH Indicator Strips; however a material safety data sheet is available.

Ideally, one method, test kit, and/or combination of test kits will be used for each protocol-specified test throughout the duration of the study. If for any reason a new or alternative method or kit must be used after study initiation, site laboratory staff must perform a validation study of the new method or test prior to changing methods. Contact the HPTN NL for further guidance on validation requirements. Similarly contact the HPTN NL in the event that the local normal range for any protocol-specified test is updated after study initiation.

Regardless of whether tests are performed in clinic or laboratory settings, study staff who perform the tests must be trained in proper testing and associated QC procedures prior to performing the tests for study purposes; training documentation should be available for inspection at any time.

When tests are performed in clinic settings, the same documentation and quality control (QC) practices required in the laboratory must be undertaken in the clinic. In-clinic testing and QC procedures must be documented on log sheets that are maintained in the clinic and reviewed by the study site Laboratory Manager (or designee) at least once per month. Once the log sheets are reviewed by the Laboratory Manager (or designee) they may then be stored in the local laboratory, if desired. In the event that proper QC procedures are not followed in the clinic, or that adequate QC is not maintained, the study site Laboratory Manager is responsible for ensuring that corrective action is taken and documented. Sample log sheets are available from the HPTN NL.

As transmission of HIV and other infectious agents can occur through contact with contaminated needles, blood, blood products, and genital secretions, all study staff must take appropriate precautions when collecting and handling biological specimens. Guidance on universal precautions available from the US Centers for Disease Control and Prevention:

[http://www.cdc.gov/ncidod/dhqp/bp\\_universal\\_precautions.html](http://www.cdc.gov/ncidod/dhqp/bp_universal_precautions.html)

Additional laboratory reference information can be found in the joint HPTN-MTN Laboratory Manual, which is available at:

<http://www.hptn.org/web%20documents/CentralLab/HPTN-MTNLABMANUALVersion1.0.pdf>

Provided in the remainder of this section is information intended to standardize laboratory procedures across sites. Adherence to the specifications of this section is essential to ensure that primary and secondary endpoint data derived from laboratory testing will be considered acceptable to drug regulatory authorities across study sites.

Questions about specimen collection should be raised with your site investigator or the HPTN Network Laboratory Deputy Director Estelle Piwowar-Manning (410-614-6736 or [epiwowa@jhmi.edu](mailto:epiwowa@jhmi.edu)).

## 10.2 HPTN 052 Required Laboratory Assays

Table 10-1 outlines all laboratory assays required by the HPTN 052 protocol. In addition, the table identifies the kit, procedure, or instrument that must be used for these protocol-specified assays when specified. These kits, procedures, and instruments are required regardless of whether they are conducted in-house or at a contract lab. **A site-specific SOP must be in place for each laboratory assay before the study can be conducted at a given site.**

**Table 10-1: HPTN 052 Required Laboratory Assays**

| Laboratory Assay               | Performance Site | Specific Kit, Procedure, or Instrument   |
|--------------------------------|------------------|--|
| <b>HIV/AIDS Related Assays</b> |                  |  |
| HIV EIA Antibody Test          | All sites        | Rapid Testing: Trinity Biotech Uni-Gold® or Oraquick®, or Bio-Rad EIA, and Western Blot Kit or alternative HIV test kit (Rapid, EIA, WB/IFA) approved by the U.S. FDA. A list of approved kits can be found online at: <a href="http://www.fda.gov/cber/products/testkits.htm">http://www.fda.gov/cber/products/testkits.htm</a> |

| Laboratory Assay  | Performance Site   | Specific Kit, Procedure, or Instrument  |
|---|--|---|
| <b>HIV/AIDS Related Assays</b>  |  |   |
| HIV Western Blot (Confirmatory)   | Sites may use either Western Blot or IFA   | Bio-Rad Western Blot Kit or alternative HIV WB or IFA test kit approved by the U.S. FDA.<br>A list of approved kits can be found online at: <a href="http://www.fda.gov/cber/products/testkits.htm">http://www.fda.gov/cber/products/testkits.htm</a> |
| HIV IFA (Confirmatory)  |  | Fluorognost HIV-1 IFA   |
| Blood plasma HIV-1 RNA PCR  | All sites  | Roche AMPLICOR™ v 1.5 Standard (microwell, COBAS, or Ampliprep)   |
| CD4+ cell count   | All sites  | Not specified   |
| HIV genotyping (regionally tested, see Section 10.8)  | Rio de Janeiro, Brazil; Pune, India; Chennai, India; Johannesburg, South Africa, HPTN NL | ViroSeq™ HIV Genotyping System  |
| <b>Safety Related Assays</b>  |  |   |
| CBC (including hemoglobin and platelets)  | All sites  | Not specified   |
| Blood chemistry (defined as sodium, potassium, chloride, phosphate, bicarbonate, creatinine, and albumin)   | All sites  | Not specified   |
| LFTs (defined as AST [SGOT], ALT [SGPT], alkaline, phosphatase, and total bilirubin)  | All sites  | Not specified   |
| Optional blood chemistries to be performed as needed (pm) defined as: lipase, creatine kinase, lactate, triglycerides   | All sites  | Not specified   |
| <b>STD Diagnosis</b>  |  |   |
| Urine PCR for Chlamydia trachomatis (CT) and Neisseria gonorrhoea (GC) for men  | All sites  | NAT (nucleic acid testing)  |
| PCR for CT and GC in women using endocervical swab or urine (NOTE: urine may be used in the ProbeTec System or GenAptima System, urine may not be used in the Roche system) | All sites  | NAT (nucleic acid testing)  |
| Syphilis serology   | All sites  | Not specified   |
| <b>Disease Diagnosis</b>  |  |   |
| Wet mount for TV, BV, Candida   | All sites  | HPTN Network Laboratory training provided   |
| Hepatitis B serology  | All sites  | Not specified   |
| <b>Pregnancy</b>  |  |   |
| Urine pregnancy test  | All sites  | Recommended, but not required: QUIDEL QuickVue hCG Urine pregnancy kit  |

| Laboratory Assay   | Performance Site | Specific Kit, Procedure, or Instrument          |
|--|------------------|---|
| <b>Assays To Be Performed by the HPTN Network Laboratory</b> |                  |   |
| Cervical swab for HIV-1 RNA                                  | HPTN NL          | Roche AMPLICOR 1.5                              |
| Semen for HIV-1 RNA  | HPTN NL          | NASBA assay or modified Roche AMPLICOR 1.5      |
| Genital ulcer swab for multiplex PCR                         | HPTN NL          | Method developed by the HPTN Network Laboratory |

### 10.2.1 HIV Antibody Testing Algorithm

Sites **must** follow the HIV antibody testing algorithm outlined in Appendix II of the protocol to determine HIV status for Partners during follow-up visits. However, sites may use their own, validated HIV antibody testing algorithm for screening as long as U.S. FDA-approved test kits are used. If an alternative HIV testing algorithm is used for screening, it must be contained in the site's HIV antibody testing (HIV EIA antibody test/Western blot/IFA) SOP and approved by the HPTN NL.

All laboratory staff who read and interpret WB results are required to complete proficiency testing approximately every six months. The HPTN NL will post an image of an actual WB run on the HPTN 052 web page for this purpose. Relevant laboratory staff from each site will review these images and submit their interpretations of the images to the HPTN NL via the web page. After each proficiency testing cycle, the HPTN NL will report results back to each site Laboratory Manager/Director and specify any corrective action that may be needed. Contact the HPTN NL for additional information and guidance on performing and documenting the proficiency testing. Also contact the HPTN NL when new laboratory staff are hired, so that proficiency testing can take place prior to such staff interpreting WBs for study purposes.

Perform all tests according to site SOPs and package inserts. All staff involved in HIV testing and verification of HIV test results should be aware of the different testing timeframes for each rapid test, so that all tests are performed and verified within the specified timeframes. Place appropriate timekeeping devices in all test settings to ensure that each test is read and verified at appropriate timepoints. Document the testing start and stop times as well as result verification times on testing log sheets.

At sites using the FDA-approved Genetic Systems WB, manufactured by Bio-Rad Laboratories, interpret results based on the pattern of bands present, as follows:

- **Positive:** At least two of the major bands — gp160/gp120, gp41, p24 — must be present and must be at least as intense as the low positive control gp120 band. The gp41 band must be broad and diffuse.
- **Indeterminate:** One or more bands are present, but the blot does not meet the criteria for a positive result as described above.

- Negative: No bands are present

### 10.3 Labeling Specimens and Recording Laboratory Results

All containers into which specimens are initially collected (e.g., urine collection cups, blood collection tubes) will be labeled with Participant ID (PTID) labels. Microscope slides used for evaluation of vaginal fluids also will be labeled with PTID labels. SCHARP will provide a program to assist in the pre-printing of the PTID labels. Study staff must write the specimen collection date on each label. The visit code also may be written on the label. When specimens are tested at the local lab, any additional labeling required for on-site specimen management and chain of custody will be performed in accordance with site SOPs. Stored plasma specimens will be entered into the Laboratory Data Management System (LDMS) and labeled with LDMS-generated cryovial labels. Genital ulcer swabs collected for Multiplex PCR testing at the HPTN NL also will be entered into LDMS and labeled with LDMS-generated cryovial labels. Vaginal fluid slides prepared for Gram stain evaluation at the HPTN NL will be entered into LDMS and labeled with LDMS-generated labels.

#### 10.3.1 Local Specimen Testing

For samples being processed and tested locally, each site may use the labeling system used for their local procedures. Document all local lab results on the appropriate DataFax Laboratory Results forms. See Section 12 for detailed forms completion instructions. If a test is not specifically required for the protocol, record the results in the participant's chart/clinical notes only.

#### 10.3.2 Remote Specimen Testing

Samples being sent to the HPTN Network Laboratory or a regional laboratory for testing will be labeled and entered into the Laboratory Data Management System (LDMS). For samples shipped to the HPTN Network Laboratory, the results will not be recorded on a DataFax form. Samples analyzed at designated regional laboratories will be submitted to a central database coordinated by the HPTN Network Laboratory.

### 10.4 The Laboratory Data Management System (LDMS)

The Laboratory Data Management System (LDMS) is used to track the collection, storage, and shipment of laboratory specimens tested at remote laboratories (the HPTN Network Laboratory or a regional laboratory) or stored for future analysis.

**NOTE:** Samples that are **not** sent to the HPTN Network Laboratory, a regional lab for HIV genotyping, or for storage should **not** be logged into LDMS.

Detailed instructions for use of LDMS are available at:

<http://www.fstrf.org/ldms/manual/5.0/manual5.0.html>.

As of the date of this section, the current version of LDMS is Version 5.6. All sites should upgrade to this version as soon as possible. All sites must use either the “LDMS1” label format or the HPTN Barcode format in order to ensure that both the Specimen ID and the Global ID assigned to each specimen are printed on LDMS-generated labels.

An example of the two-dimensional LDMS-generated barcode label is below:



500V08000009  
FEQ0043F-01  
999515640 057  
03/Jan/2005 08:00  
BLD EDT PL2 N/A  
1.00 ML 0 Scr

Row 1: LDMS Specimen ID  
Row 2: Global Specimen ID  
Row 3: Patient Identifier (ID1) and Study/Protocol Identifier (ID2)  
Row 4: Specimen Date or Harvest Date and Specimen Collection Time  
Row 5: Primary Type, Additive Type, Derivative Type, and Sub Additive/Derivative Type  
Row 6: Volume/Volume Unit and Visit/Visit Unit (VID)

Questions related to use of LDMS in HPTN 052 should be directed to Estelle Piwowar-Manning ([epiwowa@jhmi.edu](mailto:epiwowa@jhmi.edu), +410-614-6736). Please contact Leslie Cottle at SCHARP ([leslie@scharp.org](mailto:leslie@scharp.org), 206-667-7405) with questions about LDMS labels and specimen tracking sheets.

Technical support also is available from LDMS User Support. Usual business hours for LDMS User Support are 12:00 am to 6:00 pm ET on Monday through Friday. This will allow international laboratories to receive immediate support via phone or email for any LDMS issues or questions. LDMS User support will also be available via pagers from 6pm to 12 am Monday through Friday and on weekends if the site is locked out of the LDMS or experiencing errors that prevent the completion of LDMS work. Please contact LDMS User Support as follows:

Email: [ldmshelp@fstrf.org](mailto:ldmshelp@fstrf.org)

Phone: +716-834-0900, ext 7311

Fax: +716-898-7711

LDMS User Support can be paged during off business hours if you are locked out of LDMS or experience errors that prevent you from completing LDMS lab work. To page LDMS User Support, email LDMS pager 1 (address shown in table below) and include the following information in the body of your email:

- LDMS lab number (this is a three-digit number that is different from your network assigned clinical site number)

- The full telephone number at which you can be reached, including the country code and city code if you are outside the United States
- A short description of the problem

If a response is not received within 15 minutes after emailing LDMS 1, try emailing LDMS 2, then finally, LDMS 3.

The pagers also can be reached via telephone. When paging via telephone, after dialing you will hear a voice greeting followed by three quick beeps that indicate you are connected to the paging service. Please include the full telephone number at which you can be reached, including the country and city codes if you are outside the United States. Please call LDMS pager 1 first (telephone number shown in table below). If you do not receive a response within 15 minutes after calling LDMS 1, please try LDMS 2, then finally, LDMS 3.

| <b>LDMS User Support Paging Details</b> |                      |                         |
|---|----------------------|-------------------------|
| <b>Pager</b>                            | <b>Email Address</b> | <b>Telephone Number</b> |
| LDMS 1                                  | ldmspager1@fstrf.org | +716-556-0583           |
| LDMS 2                                  | ldmspager2@fstrf.org | +716-556-0584           |
| LDMS 3                                  | ldmspager3@fstrf.org | +716-556-0585           |

Each site must export its LDMS data to Frontier Science (FSTRF) on a weekly basis. Exported data are use by the HPTN SDMC to generate a monthly specimen repository report and to reconcile data entered in LDMS with data entered on study case report forms. Any discrepancies identified during the reconciliation are included in a monthly discrepancy report for each site. Sites are expected to resolve all discrepancies within two weeks of receipt of the report. The HPTN NL is responsible for reminding sites to adhere to the two-week timeframe and for following up with sites that do not resolve discrepancies within two weeks. The HPTN SDMC reviews the discrepancy reports for critical samples that appear to be missing, and works with the NL and site staff to undertake appropriate corrective action. All corrective action should be documented in paper-based clinic and/or laboratory records as appropriate, and entered in the details section of LDMS. The NL and SDMC will discuss and document any items that, although resolved, appear ‘unresolvable’ in LDMS.

#### **10.4.1 Specimen Labels and LDMS Specimen Tracking Sheets**

SCHARP will provide all sites with a specimen label program and LDMS Specimen Tracking Sheets specific for HPTN 052. Materials will be shipped on a site-by-site basis.

- **Specimen labels:** All specimen labels are pre-printed with the participant identification number (PTID) and space for clinical staff to write the visit code and specimen collection date. After the visit code and collection date are completed, labels are applied to each specimen collection tube or container in the clinic. To ensure proper adhesion, make sure the tube surface is clean, dry, and at room temperature before applying the label.
- **LDMS Specimen Tracking Sheet:** This sheet identifies those specimens that will be entered into LDMS and accompanies them to the lab. On the LDMS Specimen Tracking Sheet, record the PTID, visit code and specimen collection date. A sample LDMS Specimen Tracking Sheet for both the Index and the Partner is shown in Figure 10-1.

Specimens from a single participant are packaged together. Each package includes its own Specimen Tracking Sheet. Index specimens must be packaged and documented separately from Partner specimens.

Figure 10-1

**DO NOT FAX THIS FORM TO DATAFAX**

**HPTN 052  
Index LDMS Specimen Tracking Sheet**

Group: HPTN

Visit Code (Vst)

Index ID (PID)

-   -   -

Site Number      Index Number      Partner      Chk

Specimen Collection Date

dd      MMM      yy

Protocol #: 052

| # of TUBES (or Specimens) | PRIMARY SPECIMEN TYPE               | ADDITIVE  |
|---------------------------|-------------------------------------|---|
| <input type="checkbox"/>  | Blood (BLD)                         | <input type="checkbox"/> EDTA <input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____<br>Plasma aliquot instructions:<br><input type="checkbox"/> Lab to store at least eight (8) 1.0 mL aliquots or sixteen (16) 0.5 mL aliquots<br>Serum aliquot instructions:<br><input type="checkbox"/> Lab to store at least three (3) 1.0 mL aliquots<br>PBMC aliquot instructions:<br><input type="checkbox"/> Lab to store at least two (2) 5x10 <sup>6</sup> cryovials. Record the number of cells per vial in the volume field in LDMS with volume unit CEL. |
| <input type="checkbox"/>  | Cervical sample for HIV-1 RNA (CER) | <input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____   |
| <input type="checkbox"/>  | Semen sample for HIV-1 RNA (SEM)    | <input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____   |
| <input type="checkbox"/>  | GUD swab (GLU)                      | <input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____   |
| <input type="checkbox"/>  | Other, specify: _____               | <input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____   |

Comments: \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

Clinic Staff Initials: \_\_\_\_\_ Sending Staff      LDMS Data Entry Date:       / \_\_\_\_\_ Receiving Staff

dd      MMM      yy

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**DO NOT FAX THIS FORM TO DATAFAX**

## HPTN 052

### Partner LDMS Specimen Tracking Sheet

Group: HPTN

Visit Code (Vst)

Partner ID (PID)

.

-    -   -

Site Number      Index Number      Partner      Chk

Specimen Collection Date

/    /

dd                  MMM                  yy

Protocol #: 052

| # of TUBES<br>(or Specimens) | PRIMARY SPECIMEN TYPE               | ADDITIVE  |
|------------------------------|-------------------------------------|---|
| <input type="checkbox"/>     | Blood (BLD)                         | <input type="checkbox"/> EDTA <input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____<br>Plasma aliquot instructions:<br><input type="checkbox"/> Lab to store at least eight (8) 1.0 mL aliquots or sixteen (16) 0.5 mL aliquots<br>Serum aliquot instructions:<br><input type="checkbox"/> Lab to store at least three (3) 1.0 mL aliquots<br>PBMC aliquot instructions:<br><input type="checkbox"/> Lab to store at least two (2) 5x10 <sup>6</sup> cryovials. Record the number of cells per vial in the volume field in LDMS with volume unit CEL. |
| <input type="checkbox"/>     | Cervical sample for HIV-1 RNA (CER) | <input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____   |
| <input type="checkbox"/>     | Semen sample for HIV-1 RNA (SEM)    | <input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____   |
| <input type="checkbox"/>     | GUD swab (GLU)                      | <input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____   |
| <input type="checkbox"/>     | Other, specify: _____               | <input type="checkbox"/> No Additive <input type="checkbox"/> Other, specify: _____   |

Comments: \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

Clinic Staff Initials: \_\_\_\_\_ Sending Staff      LDMS Data Entry Date:   /    /   Receiving Staff

dd                  MMM                  yy

Version 3.0, 14-OCT-08

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## 10.4.2 LDMS Laboratory Specimen Processing

When the specimen shipment is received at the local LDMS Laboratory, each package in the shipment will be checked to ensure that the all specimens in the package contain the same ID as on the LDMS Specimen Tracking Sheet and that the type and number of each type of specimen marked on the Specimen Tracking Sheet is correct. If discrepancies are noticed, the clinic staff should be contacted

The LDMS specimen type codes that will be used for HPTN 052 are listed in Table 10-2.

**Table 10-2: LDMS Specimen Type Codes**

| Test                        | Primary | Additive   | Derivative | Sub Add/Deriv |
|-----------------------------|---------|------------|------------|---------------|
| Serum storage               | BLD     | NON or SST | SER        | N/A           |
| Plasma storage              | BLD     | EDT        | PL2        | N/A           |
| PBMC storage                | BLD     | EDT        | CEL        | DMS           |
| Genital Secretions – Female | CER     | NON        | TFS        | GIT           |
| Genital Secretions – Male   | SEM     | NON        | FLD        | N/A           |
| Swab for Multiplex          | GLU     | NON        | SWB        | N/A           |
| Whole Blood Storage         | BLD     | EDT        | BLD        | N/A           |

If specimens are designated by the protocol to be shipped either to the HPTN Network Laboratory or a regional laboratory, the specimens will be removed from the local freezer storage and placed into an appropriate shipping container using the LDMS system. Instructions for shipping samples are included in Section 10.7.

If there are any problems associated with sample aliquots such as inadequate numbers of aliquots due to a short draw, lost sample, lab error, comment the problem in the details section of the specimen and cascade the information to the aliquot level.

## 10.5 Specimen Collection

This section describes how specimens should be collected for immediate laboratory analysis or long-term storage.

### 10.5.1 Blood

Blood will be collected for testing and storage throughout this protocol. All specimen collection tubes must be labeled with a SCHARP-provided PTID label. Labeling should take place in the presence of the participant. Collect specimens and label tubes according to local regulations and site-specific SOPs. Table 10-3 shows the volume of blood, type of blood collection tube, storage requirements, and blood

test to be conducted at each visit. Refer to the protocol for the required chemistry (including liver functions tests) and hematology measurements.

### 10.5.2 Urine

Urine will be collected for pregnancy (women only), Chlamydia and gonorrhea (men only). Once LoA#2 to V. 3.0 is approved by all applicable IRB/ECs at your site, and if the Probetec or GenAptima system is used, urine can be collected and used from women for Chlamydia and gonorrhea testing. Prior to urine collection, the urine collection cup (one per participant per visit) should be labeled (participant ID label and hand-written date). Urine should be collected as follows:

- Participant should not have urinated within one hour prior to collection.
- Provide participant with a labeled screw-top urine collection cup. A line should be drawn on the urine collection cup to indicate 20 mL. (**Note:** Only 15 mL of urine is required for testing; however, it is difficult to collect such a small volume.)
- Instruct participant not to clean the penis or labia prior to collecting the urine specimen.
- Instruct participants to collect up to 20 mL (up to the line on the cup) of the first portion of urine stream (*i.e.*, first void / dirty catch urine).
- Instruct participant to screw lid on tightly.

Table 10-3 shows the type of urine test to be conducted at each visit.

**Table 10-3a:** HPTN 052 Blood and Urine Specimen Collection and Testing for those who consent to long-term storage

| Test                                       | Screening   |                 |             | Enrollment   |              | 2-Week and Month 2        | Month 1 and ART initiation (Arm 2 only) | Quarterly Visits |              | Yearly Visits |              | Partner Seroconverts |              | Confirm Virologic Failure |
|--|-------------|-----------------|-------------|--------------|--------------|---------------------------|---|------------------|--------------|---------------|--------------|----------------------|--------------|---------------------------|
|  | Index       | Index (if HIV+) | Partner     | Index        | Partner      | Index                     | Index                                   | Index            | Partner      | Index         | Partner      | Index                | Partner      | Index                     |
| <b>Tube 1: no additive (red top)</b>       | 5 mL        | 10 mL           | 5 mL        | 10 mL        | 10 mL        | 5 mL                      | 5 mL                                    | 10 mL            | 5 mL         | 10 mL         | 10 mL        |                      | 10 mL        |                           |
| HIV EIA Rapid Test/Western Blot            | X           |                 | X           |              |              |                           |   |                  | X            |               | X            |                      |              |                           |
| Blood Chemistry (including LFTs)           |             | X               |             | X            |              | X                         | X                                       | X                |              | X             |              |                      | X            |                           |
| Syphilis                                   |             |                 |             | X            | X            |                           |   |                  |              | X             | X            |                      |              |                           |
| Hepatitis B                                |             | X               |             |              |              |                           |   |                  |              |               |              |                      |              |                           |
| Serum Storage (3 mL)                       |             |                 |             | X            | X            |                           |   | X                |              | X             | X            |                      | X            |                           |
| <b>Tube 2: EDTA (lavender top)</b>         |             |                 |             | 5 mL         | 5 mL         |                           |   |                  |              |               |              |                      |              |                           |
| Whole Blood Storage (5 mL)                 |             |                 |             | X            | X            |                           |   |                  |              |               |              |                      |              |                           |
| <b>Tube 3: EDTA (lavender top)</b>         |             | 10 mL           |             | 10 mL        |              | 5 mL                      | 10 mL                                   | 10 mL            |              | 10 mL         |              | 10 mL                | 10 mL        | 10 mL                     |
| Plasma HIV-1 RNA <sup>1</sup>              |             |                 |             | X            |              |                           | X                                       | X                |              | X             |              | X                    | X            | X                         |
| CD4+ Cell Count                            |             | X               |             | X            |              |                           |   | X                |              | X             |              |                      | X            |                           |
| CBC with Differential                      |             | X               |             | X            |              | X                         | X                                       | X                |              | X             |              |                      | X            |                           |
| <b>Tube 4: EDTA (lavender top)</b>         |             |                 |             | 10 mL        |              |                           |   |                  |              |               |              | 10 mL                | 10 mL        | 10 mL                     |
| Plasma for HIV Genotyping                  |             |                 |             | X            |              |                           |   |                  |              |               |              | X                    | X            | X                         |
| <b>Tube 5: EDTA (lavender top)</b>         |             |                 |             | 10 mL        | 10 mL        |                           |   | 10 mL            | 10 mL        | 10 mL         | 10 mL        | 10 mL                | 10 mL        | 10 mL                     |
| Plasma Storage (5 mL)                      |             |                 |             | X            | X            |                           |   | X                | X            | X             | X            | X                    | X            | X                         |
| PBMC Storage (5 million cells, 2 aliquots) |             |                 |             | X            | X            |                           |   | X                | X            | X             | X            | X                    | X            |                           |
| <b>Total Blood Volume</b>                  | <b>5 mL</b> | <b>20 mL</b>    | <b>5 mL</b> | <b>45 mL</b> | <b>25 mL</b> | <b>10 mL</b>              | <b>15 mL</b>                            | <b>30 mL</b>     | <b>15 mL</b> | <b>30 mL</b>  | <b>20 mL</b> | <b>30 mL</b>         | <b>40 mL</b> | <b>30 mL</b>              |
| <b>URINE COLLECTION AND TESTING</b>        |             |                 |             |              |              |                           |   |                  |              |               |              |                      |              |                           |
| <b>Urine Collection Volume</b>             |             | <b>15 mL</b>    |             | <b>15 mL</b> | <b>15 mL</b> | <b>15 mL</b> <sup>2</sup> | <b>15 mL</b>                            | <b>15 mL</b>     |              | <b>15 mL</b>  | <b>15 mL</b> |                      |              |                           |
| Pregnancy (women only)                     |             | X               |             | X            |              | X                         | X                                       | X                |              | X             |              |                      |              |                           |
| Chlamydia (men only) <sup>3</sup>          |             |                 |             | X            | X            |                           |   |                  |              | X             | X            |                      |              |                           |
| Gonorrhea (men only) <sup>3</sup>          |             |                 |             | X            | X            |                           |   |                  |              | X             | X            |                      |              |                           |

CBC; complete blood count; LFT, liver function tests

1. Viral load testing may be performed from the blood collected for PBMC and plasma storage.

2. Urine collection is done for pregnancy testing at each monthly visit – but not the 2-Week visit.

3. Once LoA#2 to V3.0 of the protocol is approved at a study site, urine may be used for CT and GC testing, if acceptable equipment is in place (see Section 10.5.2).

**Table 10-3b:** HPTN 052 Blood and Urine Specimen Collection and Testing for those who do not consent to long-term storage

| Test                                 | Screening   |                 |             | Enrollment   |              | 2-Week and Month 2        | Month 1 and ART initiation (Arm 2 only) | Quarterly Visits |             | Yearly Visits |              | Partner Seroconverts |              | Confirm Virologic Failure |
|--------------------------------------|-------------|-----------------|-------------|--------------|--------------|---------------------------|---|------------------|-------------|---------------|--------------|----------------------|--------------|---------------------------|
|                                      | Index       | Index (if HIV+) | Partner     | Index        | Partner      | Index                     | Index                                   | Index            | Partner     | Index         | Partner      | Index                | Partner      | Index                     |
| <b>Tube 1: no additive (red top)</b> | 5 mL        | 10 mL           | 5 mL        | 5 mL         | 5 mL         | 5 mL                      | 5 mL                                    | 5 mL             | 5 mL        | 5 mL          | 5 mL         |                      | 5 mL         |                           |
| HIV EIA Rapid Test/Western Blot      | X           |                 | X           |              |              |                           |   |                  | X           |               | X            |                      |              |                           |
| Blood Chemistry (including LFTs)     |             | X               |             | X            |              | X                         | X                                       | X                |             | X             |              |                      | X            |                           |
| Syphilis                             |             |                 |             | X            | X            |                           |   |                  |             | X             | X            |                      |              |                           |
| Hepatitis B                          |             | X               |             |              |              |                           |   |                  |             |               |              |                      |              |                           |
| <b>Tube 3: EDTA (lavender top)</b>   |             | 10 mL           |             | 10 mL        |              | 5 mL                      | 10 mL                                   | 10 mL            |             | 10 mL         |              | 10 mL                | 10 mL        | 10 mL                     |
| Plasma HIV-1 RNA <sup>1</sup>        |             |                 |             | X            |              |                           | X                                       | X                |             | X             |              | X                    | X            | X                         |
| CD4+ Cell Count                      |             | X               |             | X            |              |                           |   | X                |             | X             |              |                      | X            |                           |
| CBC with Differential                |             | X               |             | X            |              | X                         | X                                       | X                |             | X             |              |                      | X            |                           |
| <b>Tube 4: EDTA (lavender top)</b>   |             |                 |             | 10 mL        |              |                           |   |                  |             |               |              | 10 mL                | 10 mL        | 10 mL                     |
| Plasma for HIV Genotyping            |             |                 |             | X            |              |                           |   |                  |             |               |              | X                    | X            | X                         |
| <b>Tube 5: EDTA (lavender top)</b>   |             |                 |             | 5 mL         | 5 mL         |                           |   |                  |             |               |              |                      |              |                           |
| Plasma Storage (5 mL)                |             |                 |             | X            | X            |                           |   |                  |             |               |              |                      |              |                           |
| <b>Total Blood Volume</b>            | <b>5 mL</b> | <b>20 mL</b>    | <b>5 mL</b> | <b>30 mL</b> | <b>10 mL</b> | <b>10 mL</b>              | <b>15 mL</b>                            | <b>15 mL</b>     | <b>5 mL</b> | <b>15 mL</b>  | <b>5 mL</b>  | <b>20 mL</b>         | <b>25 mL</b> | <b>20 mL</b>              |
| <b>URINE COLLECTION AND TESTING</b>  |             |                 |             |              |              |                           |   |                  |             |               |              |                      |              |                           |
| <b>Urine Collection Volume</b>       |             | <b>15 mL</b>    |             | <b>15 mL</b> | <b>15 mL</b> | <b>15 mL</b> <sup>2</sup> | <b>15 mL</b>                            | <b>15 mL</b>     |             | <b>15 mL</b>  | <b>15 mL</b> |                      |              |                           |
| Pregnancy (women only)               |             | X               |             | X            |              | X                         | X                                       | X                |             | X             |              |                      |              |                           |
| Chlamydia (men only) <sup>3</sup>    |             |                 |             | X            | X            |                           |   |                  |             | X             | X            |                      |              |                           |
| Gonorrhea (men only) <sup>3</sup>    |             |                 |             | X            | X            |                           |   |                  |             | X             | X            |                      |              |                           |

CBC; complete blood count; LFT, liver function tests

1. Viral load testing may be performed from the blood collected for PBMC and plasma storage.
2. Urine collection is done for pregnancy testing at each monthly visit – but not the 2-Week visit.
3. Once LoA#2 to V3.0 of the protocol is approved at a study site, urine may be used for CT and GC testing, if acceptable equipment is in place (see Section 10.5.2).
4. These are suggested blood volumes. Adjustments may have to be made if the samples are being analyzed in two different places (e.g. serology vs. chemistry as at NARI and in Rio de Janeiro).

**Table 10-4:** HPTN 052 Semen, Cervical Secretion, Vaginal/Cervical Swabs, Vaginal pH, and Genital Ulcer Specimen Collection and Testing

| Test   | Screening |                 |         | Enrollment |         | 2-Week and Monthly Visits (Month 1 and 2 only) | Quarterly Visits |         | Yearly Visits |         | Partner Seroconverts |         | Confirm Virologic Failure |
|--|-----------|-----------------|---------|------------|---------|--|------------------|---------|---------------|---------|----------------------|---------|---------------------------|
|  | Index     | Index (if HIV+) | Partner | Index      | Partner | Index  | Index            | Partner | Index         | Partner | Index                | Partner | Index                     |
| <b>SEMEN COLLECTION AND TESTING</b>                      |           |                 |         |            |         |  |                  |         |               |         |                      |         |                           |
| Seminal HIV-1 RNA  |           |                 |         | X          |         |  |                  |         | X             |         | X                    | X       |                           |
| Seminal Plasma Storage                                   |           |                 |         | X          |         |  |                  |         | X             |         | X                    | X       |                           |
| <b>CERVICAL/VAGINAL SECRETION COLLECTION AND TESTING</b> |           |                 |         |            |         |  |                  |         |               |         |                      |         |                           |
| Cervical HIV-1 RNA (Tear-Flo™ Stips)                     |           |                 |         | X          |         |  |                  |         | X             |         | X                    | X       |                           |
| Cervical Secretion Storage (Tear-Flo™ Stips)             |           |                 |         | X          |         |  |                  |         | X             |         | X                    | X       |                           |
| Vaginal Swabs for BV, TV, and Candida                    |           |                 |         | X          | X       |  |                  |         | X             | X       |                      |         |                           |
| Vaginal pH   |           |                 |         | X          | X       |  |                  |         | X             | X       |                      |         |                           |
| Endocervical swabs for Gonorrhea and Chlamydia           |           |                 |         | X          | X       |  |                  |         | X             | X       |                      |         |                           |
| <b>GENITAL ULCER DIAGNOSIS</b>                           |           |                 |         |            |         |  |                  |         |               |         |                      |         |                           |
| Swab for Multiplex PCR                                   |           |                 |         | [X]        | [X]     | [X]  | [X]              | [X]     | [X]           | [X]     | [X]                  | [X]     | [X]                       |

[ ] = to be conducted whenever clinically indicated.

### 10.5.3 Semen

Semen will be collected for specimen storage and to test for seminal HIV viral load. Table 10-4 depicts when semen samples should be collected.

Prior to semen collection, all semen collection containers should be labeled (participant ID label and hand-written date). If at all possible, semen specimens should be collected in the clinic; however, if this option is not feasible, participants may collect this specimen at home and return it to the clinic within two hours of collection. If the sample is collected at home, participants should be given the labeled semen collection container and instructions for how to collect and return the specimen prior to collection. Semen should be collected as follows:

- Provide participant with a labeled semen collection container.
- 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, ask him to pull back the foreskin before cleaning the head and opening.
- Provide the participant with a private location and instruct him to masturbate, collecting the entire specimen in the container. If the specimen is collected at home, the specimen should be returned to the clinic within 2 hours.

Semen samples must be processed by the lab within 4 hours of specimen collection (refer to Section 10.6.5).

### 10.5.4 Cervical/Vaginal Secretions

If the woman is menstruating during a visit that requires a pelvic exam, she may return before the visit window closes or for an interim visit during which the exam can be conducted and the samples collected. If this is not possible, the exam and sample collection can be conducted at the next monthly visit. If the samples are still unattainable at this subsequent monthly visit (either within the visit window or at an interim visit), study staff should not attempt to collect the samples again until the protocol requires it. Study staff should try to schedule the enrollment visit at a time when the female Index case is not menstruating as this is a critical baseline measurement that should be collected prior to ART initiation.

Done properly, the pelvic exam and related collection of samples are minimally invasive and should not induce premature labor or other complications. However, if the participant is in the second trimester of pregnancy, and if she or the clinician has concerns regarding the safety of the pelvic exam and/or the related collection of samples, the tests should not be done and study staff should be sure to document the reason in the source documentation.

#### 10.5.4.1 Samples for Cervical HIV-1 RNA

Cervical secretions will be collected for specimen storage and to test for cervical HIV viral load. Table 10-4 depicts when cervical secretion samples should be collected. This specimen should be collected before any other cervical or vaginal samples.

The participant must refrain from any kind of sexual activity, douching, and inserting any intravaginal products for at least 48 hours prior to the collection of cervical specimens.

Tear-Flo™ will be used as wicks to collect primarily cell-free virions from the endocervical canal fluid in the following manner (refer to Figure 10-2).

- Help the participant assume the lithotomy position.
- Gently insert an unlubricated and appropriately-sized speculum into the vagina and lock it into place.
- Use a large cotton-tipped swab to gently remove excessive mucus or menses clots in the vagina and the cervical os before inserting the Tear-Flo™ strips.
- Use forceps (ring or sponge forceps work well) to hold the strip on the squared end of the strip and gently insert **two** Tear-Flo™ strips simultaneously into the vagina, place through the cervical os into the distal endocervical canal and hold in place to adsorb sample. Each Tear-Flo™ strip adsorbs approximately 12µl of specimen. Adsorption usually takes approximately one minute, but may take a little longer.

Note: Only the Tear-Flo™ strip(s), and **not** the forceps should be placed in the cervical os. If a woman has a small or pinpoint cervical os (nulliparas), gently place the tip of the Tear-Flo™ strip(s) to the opening of the os and get as much secretion as possible. The strip may also be folded vertically to make it thinner and, thus, able to pass through the cervical os.

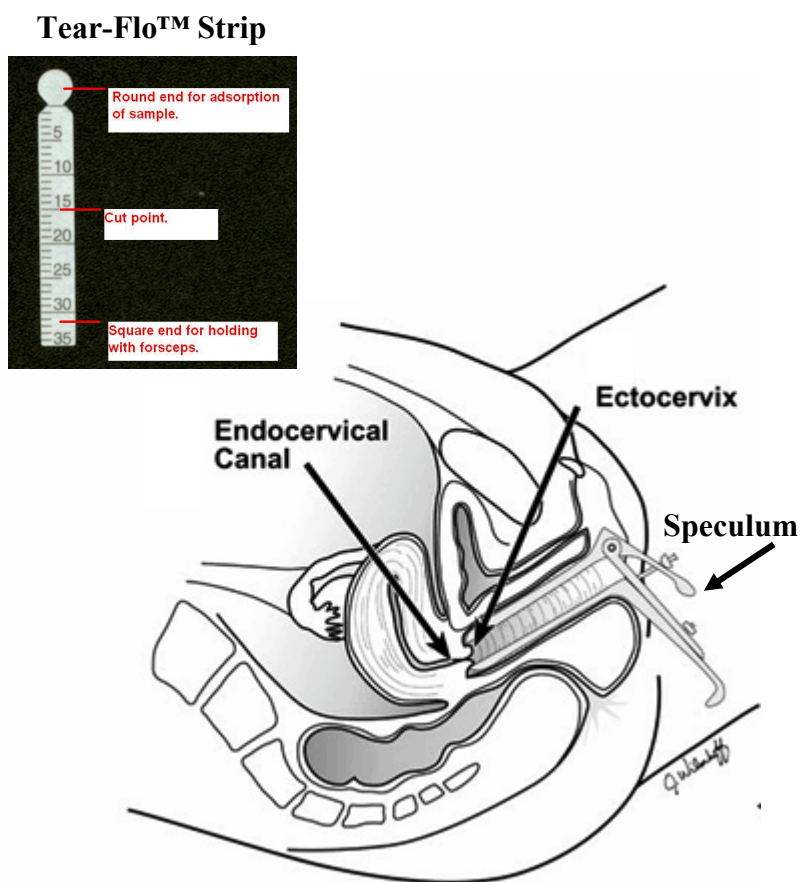
- Hold the round end of the two strips over and slightly inside one labeled plastic transport tube (1.5 or 2.0-mL cryovial) containing 500 µL of NASBA 1x Nucleic Acid Sequence Based Amplification (NASBA) lysis buffer. Cut the strips at the “15” mark with scissors, allowing the round end to fall into the cryovial tube containing buffer. Cap and invert the sample or vortex it for 5 seconds.

Note: If the Tear-Flo™ strips are to be stored in NASBA lysis buffer, the buffer must be crystal free before you begin. Most crystals will dissolve by placing the lysis buffer tube at room temperature for a few hours. If crystals remain in the tube, it should be vortexed until the crystals are gone.

- Two sets of specimens (2 Tear-Flo™ strips each) should be collected: one for specimen storage and the other for cervical HIV viral load.
- Tear-Flo™ strips in NASBA are stable for 24 hours at room temperature (~25°C) or for 14 days at 2-8°C. Long term storage should be at -70°C. Keep the sample refrigerated or on ice until it is frozen at -70°C. DO NOT store specimens in lysis buffer at -20°C.

Ordering information for Tear-Flo™ strips and NASBA lysis buffer is located in Section 10.9.

**Figure 10-2: Using Tear-Flo™ Strips to Collect Cervical Secretions.**



#### 10.5.4.2 Samples for bacterial vaginosis (BV), Trichomonas vaginalis (TV), Candida, gonorrhea, and Chlamydia

Two swabs should be taken to test for BV, TV, Candida, gonorrhea, and Chlamydia from all female participants at enrollment and at every yearly visit. Samples should also be taken for these STDs at any visit if it is clinically indicated.

The swab provided with the gonorrhea/Chlamydia diagnostic kit should be used to collect a sample from the endocervical canal. Insert the swab into the endocervical canal and wait at least 10 seconds. The swab should be placed in the vial provided with the kit and sent to the local laboratory for analysis.

A cotton-tipped swab should be gently rubbed against the vaginal walls for the first specimen. This specimen should be used to prepare a saline wet mount slide (BV, TV, and Candida detection) and a potassium hydroxide (KOH) wet mount slide (BV and Candida detection).

#### 10.5.4.3 Vaginal Wet Mount and pH

Vaginal pH will be assessed as part of on-site evaluations for bacterial vaginosis. S/P pH Indicator Strips must be used at all sites, as follows:

- During pelvic examination, touch a pH indicator strip to the vaginal wall just until the paper is moistened. Avoid contact with cervical mucus, which has a high pH. Alternatively, vaginal fluids may be collected via swab and then swabbed onto the pH strip (instead of inserting the pH strip into the vagina).
- Match the resulting color of the indicator strip to the color scale provided with the strips to determine the pH value.
- Record the pH value directly onto the appropriate case report form. It is not necessary to record pH values onto laboratory log sheets or other source documents prior to recording values onto case report forms.

Wet mount procedures for this study consist of two different preparations — saline prep and potassium hydroxide (KOH) prep — for diagnosis of bacterial vaginosis, trichomoniasis, and candidiasis, as summarized in Table 10-5.

**Table 10-5: Summary of Wet Prep Assessments and Diagnostic Criteria**

| Assessment | Saline Prep     | KOH Prep                               |
|------------|-----------------|--|
| Whiff test | Not applicable. | Positive if fishy amine odor detected. |

| Assessment   | Saline Prep  | KOH Prep  |
|--------------|--|---|
| Clue cells   | Individual cells rather than clusters of cells should be examined. Positive if at least 20% clue cells observed. Cells must be completely covered with bacteria ( <i>Gardnerella vaginalis</i> ) to be counted as clue cells.                                  | Not applicable (clue cells are lysed by KOH).           |
| Trichomonads | Positive if at least one motile trichomonad is observed. Actively motile organisms are easily seen upon low power (10X). High power (40X) may be needed to detect less vigorously motile organisms when only the flagella may be moving.                       | Not applicable (organisms are lysed by KOH).            |
| Yeast        | Positive if pseudohyphae and/or budding yeast are observed. Pseudohyphae and budding yeast may be obscured by epithelial cells. These cells will be lysed by KOH, thus pseudohyphae and budding yeast not observed in saline prep may be observed in KOH prep. | Positive if pseudohyphae or budding yeast are observed. |

Note: Bacterial vaginosis will be diagnosed based on the presence of any three of the following Amsel's criteria: homogenous vaginal discharge, vaginal pH greater than 4.5, positive whiff test, at least 20% clue cells.

Prepare and examine wet prep slides according to study site SOPs as follows:

- Use a pencil to write the PTID and specimen collection date on one side of the frosted end of two microscope slides. Affix a SCHARP-provided PTID label to the other side of the slides (on the frosted end, under the pencil markings) and write the specimen collection date in ink on each label.
- Immediately following collection from the lateral vaginal wall via swab, smear vaginal fluid specimens onto each slide. Alternatively, the swab may be placed in a glass or plastic tube with approximately six drops (100 µL) sterile physiologic saline to allow for non-immediate slide

preparation. In this case, vaginal fluid specimens should be smeared onto the two slides upon receipt from the collecting clinician.

- Apply one drop of 10% KOH to one slide and immediately perform whiff test for a “fishy” amine odor. Then apply coverslip.
- Apply one drop of sterile physiologic saline to the second slide, emulsify with the vaginal fluid specimen, then apply coverslip. Examine immediately at 10X magnification for epithelial cells, motile trichomonads, budding yeast, and pseudohyphae. Examine at 40X magnification to determine whether observed epithelial cells are clue cells and quantitate the cells. Clue cells are irregularly bordered squamous epithelial cells that are completely covered with bacteria (*Gardnerella vaginalis*). Clue cells must comprise at least 20 percent of the observed epithelial cells in order for the saline prep to be considered positive for clue cells.
- Examine the KOH slide at both 10X and 40X magnification for yeast and pseudohyphae.

If wet prep slides are read in-clinic by clinical staff, results may be recorded directly onto appropriate case report forms. If slides are read by lab staff (either in the local laboratory or a designated in-clinic lab area), results must be recorded onto laboratory log sheets or other laboratory source documents and then transcribed onto appropriate case report form.

#### Microscope Examination:

- Adjust the microscope to obtain maximum contrast. This can be achieved with many microscopes by racking down the condenser and lowering the light.
- Do not use immersion oil.
- Saline preparation: read before KOH. Examine under low power (10X or 20X) to focus and detect rapidly moving trichomonads or large pseudohyphae. Then examine on high dry (40X or 45X) to evaluate the presence or absence of PMNs, "clue" cells, trichomonads, yeast buds, or pseudohyphae.
- KOH preparation: Scan for pseudohyphae on low power. Confirm presence of pseudohyphae and locate yeast buds on high dry.

#### Interpretation - Vaginal Wet Mount:

##### ***Trichomonas vaginalis* vaginitis:**

Trichomonads are only seen in the saline preparation; they are lysed by

KOH. Actively motile trichomonads are easily seen on low power (10X). High power (40X) is necessary to detect less vigorously moving organisms when only the flagella may be moving.

**Table 10-6:** Positive Indicators for TV

| Saline Wet Mount                | KOH Wet Mount          |
|---------------------------------|------------------------|
| ≥ 1 actively moving trichomonad | Trichomonads are lysed |
| Numerous PMNs are often present | PMNs are lysed         |

The following errors in technique will decrease the sensitivity of the wet mount for detection of *T. vaginalis*:

- Collection of the specimen from the endocervix
- Use of cool saline
- Delay in reading the smear
- Contamination of the saline prep with KOH
- "Sloppy" preparation with too much saline, causing organisms to move rapidly across the field
- Preparation too thick
- Failure to read the slide with adequate microscope light contrast
- Examination of only a small area of the slide

**Yeast Infection:**

**Table 10-7:** Positive Indicators for Yeast Infection

| Saline Wet Mount                               | KOH Wet Mount                     |
|--|-----------------------------------|
| Pseudohyphae & budding yeast sometimes visible | Pseudohyphae & budding yeast seen |
| Epithelial cells may obscure yeast             | Epithelial cells are lysed        |
| PMNs may/may not be seen                       | PMNs are lysed                    |

**Bacterial Vaginosis:**

Individual squamous cells rather than clusters of squamous cells should be examined. A diagnosis of bacterial vaginosis requires that at least three of four Amsel's criteria be met. These criteria include: homogeneous adherent

vaginal discharge, clue cells, pH greater than 4.5 and an amine odor after addition of KOH. It should be noted that discharge that is associated with BV is non-viscous homogeneous, white, slightly watery, uniformly adherent to the vaginal walls. The key difference between normal discharge and that associated with BV is that the consistency is more uniform, watery, and less viscous than normal discharge.

**Table 10-8:** Positive Indicators for BV

| <b>Saline Wet Mount</b>            | <b>KOH Wet Mount</b>   |
|------------------------------------|------------------------|
| Numerous "clue" cells <sup>a</sup> | "Clue" cells are lysed |
| Few or no PMNs                     | PMNs are lysed         |

<sup>a</sup> An irregularly bordered squamous epithelial cell with at least 75% of its outline obliterated by sheets of small bacteria.

#### 10.5.4.4 Certification for Wet Mount

Prior to study initiation, the HPTN Network Laboratory (now the MTN NL) conducted on-site training and proficiency testing for clinic and laboratory staff designated to perform wet mounts. CLIA regulations require semi-annual proficiency testing; therefore site Laboratory Managers must ensure that all staff designated to perform wet mounts complete additional on-site proficiency testing approximately every six months. The MTN NL will post images of wet mount slides on their webpage with a redirect for the HPTN 052 web page for this purpose. Relevant laboratory staff from each site will review these images and submit their interpretations of the images to the MTN NL via the web page. After each proficiency testing cycle, the MTN NL will report results back to each site Laboratory Manager and specify any corrective action that may be needed. Contact the MTN NL for additional information and guidance on performing and documenting the proficiency testing. Also contact the MTN NL when new staff are hired, so that appropriate training can take place prior to such staff performing wet mounts for study purposes.

#### 10.5.5 Swab for Multiplex PCR

Genital ulcers observed during follow-up will be sampled for multiplex PCR testing at the HPTN NL for chancroid, HSV-2, and syphilis. Instructions for specimen collection, preparation, storage, and shipment are as follows:

- Swab the base of each observed ulcer using a plastic shaft Dacron swab. If a cluster of ulcers is observed, sample each ulcer in the cluster with the same swab. Otherwise use a different swab for each ulcer.

- Immediately place each swab in a 2 mL cryovial labeled with a SCHARP-provided PTID label. Break off the end of the swab to allow closure of the cryovial and securely attach the cap.
- Place the cryovial(s) in a plastic ziplock biohazard bag and immediately place the bag in a refrigerator or a cooler with an ice pack. If necessary, the cryovials(s) may be stored refrigerated for up to 24 hours prior to freezing. Do not store at -20°C.
- Deliver the cryovial(s) and an LDMS Specimen Tracking Sheet to the local LDMS laboratory.
- Using the LDMS Specimen Tracking Sheet, log the cryovial(s) into LDMS (specimen type = GLU) and generate an LDMS cryovial label for each tube. Affix the LDMS label to the cryovial (over the SCHARP-provided PTID label).
- Store the cryovial(s) in the freezer locations assigned in LDMS at -70° C.
- Ship the cryovials(s), frozen, to the HPTN NL at Johns Hopkins University as part of the next routine shipment of plasma samples. Detailed instructions for preparing the shipment in LDMS are available at:

[http://www.hptn.org/research\\_studies/HPTN052Lab.asp](http://www.hptn.org/research_studies/HPTN052Lab.asp)

## 10.6 HPTN 052 Required Stored Specimens

Throughout the course of HPTN 052, plasma, serum, whole blood, peripheral blood mononuclear cells (PBMC), semen, and cervical secretions will be collected and stored for future analysis. For participants who do not consent for long-term specimen storage and possible future research testing, archived samples will be destroyed after all protocol-required and quality assurance testing has been completed. Destroyed specimens should be deleted from LDMS. After the study is completed, the SDMC will provide each site with a list of participants who did not consent to indefinite storage and possible future research testing and the HPTN NL will provide detailed instructions for specimen destruction and documentation thereof.

All sites have established SOPs for weekly reconciliation and verification of all archived specimens, including plasma, serum, whole blood, PBMCs and genital secretions; these SOPs must be followed throughout the study. In the event that the required volume or number of archived aliquots is not obtained at any study visit, designated site clinic and lab staff must have an SOP in place on how frequently they will inform the HPTN CORE, SDMC and NL. The HPTN CORE, SDMC, and NL will provide guidance on how to respond to the problem. In addition to following this guidance, designated site clinic and lab staff will

work together to document the problem, take appropriate corrective and preventive action, and document all action taken.

Tables 10-3a ,10-3b and 10-4 outline when samples for storage will be collected. The following sections describe the processing and storage for each type of specimen.

For participants who do not consent to long term storage, blood still needs to be stored for QA/QC purposes from each participant in these cases. For those who do not consent to long-term storage, this blood will be discarded at the end of the study. (Refer to Table 10-3b)

Please note the suggested blood volumes are outlined in Table 3A and 3B, but adjustments may have to be made if the samples are being analyzed in two different places (e.g. serology vs. chemistry as at NARI and in Rio de Janeiro).

### **10.6.1 Whole Blood**

Whole blood is being stored as a source of DNA for future analyses. Each sample should be collected in a lavender-top tube (EDTA). No further processing of the sample is required.

- Log samples into LDMS and generate LDMS labels.
- Make as many 1mL aliquots as possible using labeled [LDMS generated label] cryovials.
- Store whole blood aliquots in a -70°C (Ultra Low) freezer within 30 hours of collection. If you have any aliquots stored at -20°C, move them to a -70°C (Ultra Low) freezer and document these changes in the LDMS.

### **10.6.2 Serum**

- Process blood for serum samples within 24 hours according to local site SOPs.
- Log samples into LDMS and generate LDMS labels.
- Make three (3) 1mL serum aliquots using labeled [LDMS generated label] cryovials.
- Store serum aliquots in at -70°C.

### **10.6.3 Plasma**

Archived plasma and PBMCs samples will come from the same 10 mL blood sample collected using an EDTA (lavender top) tube (see Table 10-3). Plasma collected for

HIV genotyping will come from a separate EDTA (lavender top) tube (see Table 10-3). Blood collected for HIV genotyping and generic plasma storage should be processed within 6 hours of sample collection. If this is not possible, then the blood should be processed within 30 hours and the time to processing should be noted in LDMS. PBMC processing should occur within 30 hours of sample collection.

- Centrifuge tube at 400 x g for 10 minutes to separate cells and plasma.
- Carefully remove plasma and avoid disturbing the cell layer. Transfer the plasma to another sterile centrifuge tube.
- Centrifuge plasma again at 800 x g for 10 minutes to remove any contaminating debris, cells, or platelets.
- Log samples into LDMS and generate LDMS labels.
- Make four (4) 1 mL or eight (8) 0.5 mL plasma aliquots using labeled [LDMS generated label] cryovials per 10 mL tube. There should be a total of eight (8) 1 mL or sixteen (16) 0.5 mL aliquots for the two 10 mL tubes. Four aliquots should be designated for HIV genotyping.

Note: Samples stored for HIV genotyping and general storage (archive) may only be used at the direction of SCHARP or the NL. If the study team wishes to perform HIV genotyping or any other tests for clinical management, additional samples must be collected from the study participant. Samples collected for HIV genotyping must be stored until the site receives a request from SCHARP to ship the samples to an HPTN NL-designated HIV genotyping testing lab.

- Store plasma aliquots at  $-70^{\circ}\text{C}$  (Ultra Low)

Note: Sample collection and storage for HIV genotyping and general storage at the confirmed failure visit must occur at the first study visit after the initial failure visit. This is the same visit at which a sample is drawn and tested for viral load to confirm treatment failure. Samples for HIV genotyping and storage must be collected and stored at this visit, even though the confirmatory viral load result is not yet available.

#### 10.6.4 PBMC

Archived plasma and PBMCs samples will come from the same 10 mL blood sample collected using an EDTA (lavender top) tube (see Table 10-3). The blood should be processed within 30 hours of sample collection. Either the Ficoll-Hypaque Underlay or Overlay Methods (density-gradient centrifugation techniques) will be used to process the PBMC sample. Refer to the Joint HPTN-MTN laboratory manual for instructions on processing PBMCs following the Cross-network SOP.

<http://www.hptn.org/web%20documents/CentralLab/HPTN-MTNLABMANUALVersion1.0.pdf> pages 413-461

or HANC website : <http://www.hanc.info/Pages/index.aspx>

Training material on PBMC processing may be found on the following website.

<http://www.ccghe.org/pbmc/player.html>

- Log samples into LDMS and generate LDMS labels.
- Make as many  $5 \times 10^6$  PBMC aliquots (1 mL) as possible using labeled [LDMS generated label] cryovials. If there are not enough cells to make a second or third  $5 \times 10^6$  cell aliquot, save the remainder of the cells in a cryovial and record the volume.

### 10.6.5 Semen

- After the semen specimen is collected, allow it to sit for at least 30 minutes, but not more than 4 hours, prior to processing to facilitate liquefaction of the semen.
- Centrifuge the sample at 800 x g for 15 minutes. Use a pipette to remove seminal plasma.
- Log samples into LDMS and generate LDMS labels.
- Make as many 0.5 mL seminal plasma aliquots as possible using labeled [LDMS generated label] cryovials. Store the volume even if it is less than 0.5mL.
- Store seminal plasma aliquots at  $-70^{\circ}\text{C}$ .

### 10.6.6 Cervical Secretions

- Vortex the cryovial containing the Tear-Flo™ strips for 5 seconds. Do not remove the Tear-Flo™ strips from the cryovial.
- Log sample into LDMS and generate LDMS label. Place label on cryovial containing the sample.
- Store cervical secretion sample at  $-70^{\circ}\text{C}$ . **Do not** store these samples at  $-20^{\circ}\text{C}$ .

## 10.7 Shipping Samples To Be Analyzed Remotely

All specimens sent to the HPTN Network Laboratory or a regional laboratory for analysis will be transported in accordance with the International Air Transport Association (IATA) specimen shipping regulations and individual carrier guidelines.

### 10.7.1 Shipping Samples to the HPTN Network Laboratory

Throughout the course of HPTN 052, the following types of samples will be shipped to the HPTN Network Laboratory: cervical Tear-Flo™ strips for HIV-1 RNA, seminal plasma for HIV-1 RNA, and genital ulcer swab for multiplex PCR.

International sites should send these samples to the HPTN Network Laboratory whenever they accumulate 15 specimens. U.S. sites may send these samples in real time. Different types of samples can be sent in the same shipment to the HPTN Network Laboratory.

Protocol Section 9.3 describes the HIV testing that the HPTN NL will perform on archived plasma for quality control and quality assurance purposes. Each site will ship plasma samples to the NL on a routine basis throughout the study, and the SDMC will provide a listing of samples (by PTID and specimen collection date) to be included in each shipment.

Upon receipt of each listing from the SDMC:

- Contact the HPTN NL at Johns Hopkins University (Estelle Piwowar-Manning: [epiwowa@jhmi.edu](mailto:epiwowa@jhmi.edu), +410-614-6736) to coordinate the timing and logistics of the shipment. The US site may ship to the HPTN NL via Federal Express Monday through Thursday, with 24-hour fax notification. For non-US sites, the HPTN NL will work with each site to arrange for shipping with World Courier.
- Working from the SDMC list of specimens to be shipped, use LDMS to generate a shipping manifest, box map, and LDMS shipping diskette for the selected samples.
- Obtain the selected specimens (one aliquot for each PTID and date) from the freezer and confirm the PTID, global ID, and date on the cryovial labels.
- Place the aliquots in a 5x5 or 9x9 cryovial box in the order of the shipping manifest.
- When shipping on carbon dioxide and/or liquid nitrogen (LN2), wrap the cryovial box in absorbent material and place it inside a shipping bag. Seal the bag and then place it in a shipping box. Fill the box with sufficient carbon dioxide (dry ice) to last at least 48 hours. World Courier will replenish dry ice as necessary. Please check with the manufacturer of the LN2 shipper for appropriate internal packaging. LN2 shippers are manufactured to maintain temperatures for 7-14 days, and World Courier should deliver the LN2 shipper within this time frame.
- Include a copy of the shipping manifest, box map, LDMS diskette, and CDC import permit in the shipment. For dry ice shipments and LN2

shipments, use diagnostics packing code 650, UN 3373. Use Non-Flammable Gas labels, Keep Upright stickers, and Do Not Drop – Handle With Care stickers, and address the shipment to:

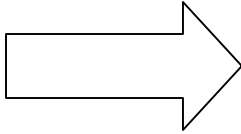
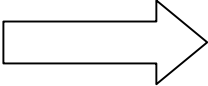
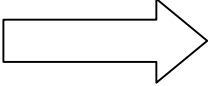
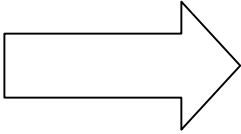
Estelle Piwowar-Manning/Dr Brooks Jackson  
Johns Hopkins University Hospital  
Department of Pathology  
Pathology Building, Room 313  
600 North Wolfe Street  
Baltimore, MD 21287  
USA

- Notify the HPTN NL via email ([epiwowa@jhmi.edu](mailto:epiwowa@jhmi.edu)) when the shipment has been picked up from the site by the courier/shipping company. Attach an electronic copy of the shipping manifest and LDMS batch to the email notification, and include the following information in the notification: name of courier/shipping company, shipment tracking number, number of boxes shipped, date of shipment, and expected date of arrival.

### **10.7.2 Shipping Samples Regionally**

Samples collected for HIV genotyping will be analyzed regionally. Table 10-9 shows where samples from each site will be sent.

**Table 10-9: Regional Locations for HIV Genotyping**

| Performance Site   |  | Regional Resistance Testing Laboratory                        |
|--|--|---|
| YRGCARE, Chennai, India (11701)  |   | YRGCARE, Chennai, India (11701)                               |
| RIHES, Chiang Mai, Thailand (31458)                                      |  |   |
| NARI, Pune, India (11601)  |   | NARI, Pune, India (11601)                                     |
| Clinics in Rio de Janeiro and Porto Alegre, Brazil (12101, 30279, 12201) |   | FIOCRUZ, Rio de Janeiro, Brazil (12101)                       |
| Parirenyatwa Hospital, Harare, Zimbabwe (30313)                          |  | University of Witwatersrand, Johannesburg, South Africa 11101 |
| Lilongwe Central Hospital, Lilongwe, Malawi (12001)                      |  |   |
| Queen Elizabeth Central Hospital, Blantyre, Malawi (30301)               |  |   |
| Witwatersrand Clinical Research Site, Johannesburg, South Africa (11101) |  |   |
| Gaborone Prevention/Treatment Trials CRS Botswana (12701)                |  |   |
| Soweto HPTN CRS Johannesburg, SA (31610)                                 |  |   |
|  |  |   |

**Note:** All labs will send HIV genotyping samples to the HPTN Network Laboratory for QA/QC testing (see Section 10.7.1).

### 10.7.2.1 Shipping Samples to YRG CARE, Chennai, India

HPTN sites located in Thailand should send plasma samples for HIV genotyping to YRG CARE located in Chennai, India. Prior to shipment, please contact Dr. P. Balakrishnan (email: [bala@yrgcare.org](mailto:bala@yrgcare.org)) or at 91-44-22542929 (phone) / 91-44-22542939 (fax) to set up the shipment.

**SHIPPING ADDRESS: Attn: Dr.P.Balakrishnan  
VHS-YRG CARE Infectious Diseases  
Laboratory,  
Admin Bldg., II-Flr, VHS, Tidel Park Road,  
Chennai - 600113.**

#### **10.7.2.2 Shipping Samples to FIOCRUZ, Rio de Janeiro, Brazil**

HPTN sites located in Brazil should send plasma samples for HIV genotyping to the Oswaldo Cruz Foundation (FIOCRUZ) located in Rio de Janeiro, Brazil. Prior to shipment, please contact Jose Carlos Couto-Fernandez at 55-21-3865-8106 or 55-21-3865-8130 (phone), 55-21-2290-0479 (fax), or [coutofer@ioc.fiocruz.br](mailto:coutofer@ioc.fiocruz.br) to set up the shipment.

**SHIPPING ADDRESS: Oswaldo Cruz Foundation (FIOCRUZ)  
Department of Immunology - IOC  
Laboratory of AIDS & Molecular Immunology  
c/o Jose Carlos Couto-Fernandez  
Leonidas Deane Building  
4th floor, Rooms 413-415  
Avenida Brasil 4365 – Manguinhos  
CEP 21045-900  
Rio de Janeiro - RJ - Brasil**

#### **10.7.2.3 Shipping Samples to Johannesburg, South Africa**

HPTN sites located in Malawi, Zimbabwe, South Africa and Botswana should send plasma samples for HIV genotyping to the University of Witwatersrand located in Johannesburg, South Africa. Prior to shipment, please contact Ischell Doddameade ([ischell.doddameade@nhls.ac.za](mailto:ischell.doddameade@nhls.ac.za)), Garth Swartz ([garth.swartz@nhls.ac.za](mailto:garth.swartz@nhls.ac.za)) and Odette Lazarus ([odette.lazarus@nhls.ac.za](mailto:odette.lazarus@nhls.ac.za)) to set up the shipment.

#### **PROCEDURE:**

1. Contact CLS to coordinate the timing and logistics of the shipment.  
Contact details:  
Odette Lazarus – Project Manager – [Odette.lazarus@nhls.ac.za](mailto:Odette.lazarus@nhls.ac.za)  
Ischell Doddameade – Logistics Manager -[ischell.doddameade@nhls.ac.za](mailto:ischell.doddameade@nhls.ac.za)  
Garth Swartz – LDMS Supervisor – [garth.swartz@nhls.ac.za](mailto:garth.swartz@nhls.ac.za)
2. Site may ship to CLS from Monday to Thursday. Never before a South Africa public holiday.
3. Place the aliquots in 9 x 9 cryovial cardboard box in the order of the shipping manifest.
4. Include a copy of the shipping manifest, box map, LDMS diskette, and export and import permits.
5. Package according to IATA regulations.

6. Address shipment to:  
Oddette Lazarus/ Ischell Doddameade / Garth Swartz  
Contract Laboratory Services  
4<sup>th</sup> Floor Spencer Lister Building  
NHLs Complex  
De Korte Street  
Braamfontien  
Johannesburg  
South Africa
7. Notify CLS via email when shipment has been picked up from the site by the courier company. Attach an electronic copy of the shipping manifest and LDMS batch, name of courier, waybill number, number of boxes, date of shipment and expected date of arrival.

### **10.7.3 International Air Transport Association (IATA) Shipping Regulations**

All frozen samples being shipped for HPTN 052 fall under the category of “diagnostic specimens” and do not need to be shipped as infectious or dangerous goods. However, dry ice and liquid nitrogen are considered to be hazardous materials. Each lab should have the current IATA manual and any person who handles, transports, receives or ships samples must be certified in IATA regulations. This certification needs to be renewed every two years as well as reviewed annually.

## **10.8 HIV Genotyping**

HIV genotyping will be performed using samples collected at selected study visits for a subset of study subjects, as described below. HIV genotyping will be performed for subjects with confirmed treatment failure, and for a subset of non-failing subjects. HIV genotyping will also be performed for Index-Partner pairs after documented seroconversion in the Partner. The HPTN NL is responsible for determining which samples should be tested. SCHARP is responsible for providing sites with instructions on which samples to ship for testing. **DO NOT** send any samples for HIV genotype testing until instructed to do so by SCHARP.

### **10.8.1 HIV Genotyping for Subjects with Confirmed Treatment Failure**

For each Index case with confirmed treatment failure, two plasma samples will be requested for genotyping: (a) a sample from the visit at which failure was confirmed (if not available, then the preceding study visit), and (b) a sample from the enrollment (pretreatment) visit. In addition, enrollment samples from a subset of non-failure subjects will be requested for genotyping at the end of the study. HIV genotyping for subjects with confirmed treatment failure and from the selected subset of non-failure subjects will be genotyped at regional resistance testing laboratories (see Table 10-9). CTUs will receive instructions from

SCHARP indicating which samples are to be shipped. Shipments will occur 2-4 times per year.

Once a CTU receives shipping instructions from SCHARP, the selected samples should be shipped to the regional resistance testing laboratory indicated in Table 10-9. Sites should send only one 1 mL or two 0.5 mL aliquots from the same sample per shipment. This will insure that back-up samples exist if there is a problem with shipping or testing. The HPTN Network Laboratory may change the assignment of regional testing laboratories during the course of the trial based on laboratory workload, capacity, cost, performance, or other factors. The Network Laboratory may request shipment of additional samples during the course of the trial.

### **10.8.2 HIV Genotyping for Subjects with Confirmed Seroconversion and Their Partners**

For each subject with confirmed seroconversion, three plasma samples will be requested for genotyping: (a) a sample from the seroconverting Partner collected at the visit at which seroconversion was documented, (b) a sample from the corresponding Index case from the same study visit (if not available, then from the preceding visit closest in time to the documentation of seroconversion), and (c) an enrollment (pre-treatment) sample from the same Index case. These samples will be analyzed at the HPTN Network Laboratory. This analysis will include HIV genotyping for antiretroviral drug resistance and subtype determination, and may also include further genotypic and phenotypic characterization of the HIV in these samples. In addition to the plasma samples, genital secretions from the Index and Partner may be requested. CTUs will receive instructions from SCHARP indicating which samples are to be shipped to the HPTN Network Laboratory. Shipments will occur 2-4 times per year, and may be combined with shipment of other QC samples.

Once a CTU receives shipping instructions from SCHARP, the selected samples should be shipped to the HPTN NL. Sites should send only two 1 mL or four 0.5 mL aliquots from the same plasma sample per shipment. This will insure that back-up samples exist if there is a problem with shipping or testing. The Network Laboratory may request shipment of additional samples during the course of the trial.

### **10.9 Local Laboratory Monitoring**

The DAIDS Clinical Site Monitoring Group (PPD) conducts quarterly monitoring visits to HPTN study sites with ongoing studies (see also Section 16 of the HPTN Manual of Operations). In addition to performing monitoring tasks specified by the Division of AIDS (DAIDS) in study clinics and administrative locations, monitors also will perform monitoring tasks specified by DAIDS in each site's local laboratory or laboratories. Laboratory monitoring tasks may include inspection of laboratory facilities and documentation as well as confirmation of

the use of LDMS and verification of specimen storage as recorded in LDMS. Specimens selected for on-site verification generally will not be pre-announced to site staff. The DAIDS Clinical Site Monitoring Group (PPD) lab division also conducts annual monitoring visits to laboratories with ongoing studies.

### **10.10 Laboratory Quality Control (QC) Reports**

Each site is responsible for submitting monthly laboratory quality control (QC) reports for their safety laboratory tests to the HPTN NL. These reports should contain the site's monthly Levey Jennings plots and corrective actions, if applicable. Contact Estelle Piwovar-Manning ([epiwowa@jhmi.edu](mailto:epiwowa@jhmi.edu)) to determine the required content and format of your site's report.

### **10.11 Laboratory Quality Assurance (QA) Testing**

The HPTN NL plans to redo HIV antibody testing in the following cases:

- 10% of all enrolled participants
- All HIV antibody-positive seroconversion specimens along with the enrollment sample for each seroconverted participant
- An equal number of HIV antibody-negative specimens along the enrollment sample for each specified participant
- Additional samples will be tested if any discrepancies are found.

The HPTN NL may also request samples to redo viral load testing based on each site's viral load proficiency status. Generally samples for QA testing will be requested from the NL on a quarterly basis.

### **10.12 Reagent and Supply Ordering Information**

All sites must maintain an adequate inventory of test kits they have selected and validated for use in HPTN 052. Kit inventories should be monitored closely and re-supply orders placed at least 8-12 weeks in advance of actual need (or longer if needed per site procurement policies and procedures). All sites are required to report their kit inventories, including kit lot numbers, to the HPTN NL on a monthly or bi-monthly basis. Notify the NL immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken.

### 10.12.1 Tear-Flo™ Strips Ordering Information

TearFlo™ Tear Test Strips may be obtained by calling Wilson Ophthalmic at 1800-222-2020 (toll free phone number for U.S. sites) or 1-405-376-9114 (phone number for international sites), faxing them at 405-376-9133, or by setting up an account through their website ([www.hilco.com](http://www.hilco.com)). The catalog number for the product is #060-0000002-00, there are 100 strips per box, and the price is \$14.95 per box (price quote as of 15 August 2005). A discount is available if 10 boxes or more are purchased at one time.

### 10.12.2 NASBA Lysis Buffer Ordering Information

NASBA lysis buffer (also called NucliSens Lysis Buffer) can be ordered from bioMérieux ([www.biomerieux.com](http://www.biomerieux.com), 800-345-8151); the website lists local distributors in Brazil, India, Thailand, West Africa, and the United States. The HPTN NL recommends that the 500 mL bottle of NASBA buffer be ordered and that 500 µL aliquots be made from it. The product number for the 500 mL bottle of NASBA buffer is 284135. Some of the international sites are not able to order this catalog number and may have to order 200292.

### 10.12.3 Fetal Bovine Serum Ordering Information

Bottles (500 ml) of validated heat inactivated Fetal Bovine Serum (FBS) have been placed on reserve with Gemini Bio-Products ([www.gembio.com](http://www.gembio.com)). The reserve lots of FBS have been validated by the DAIDS-sponsored Virology Quality Assurance (VQA) Laboratory for use in culture experiments and the analysis results are available on the web at <http://aactg.s-3.com/vqareports.htm>. The company's certificate of analysis of each lot is available upon request.

Please have the following information ready when placing your order:

Phone: 1-800-543-6464, Ext. 104 for customer service (for U.S. sites)

1-530-668-3636 (for international sites)

Fax: 1-530-668-3630

Quotation number

Customer number

Lot number

The most current quotation number, customer number, and lot number, as well as other information related to fetal bovine serum, can be found at [http://www.hptn.org/research\\_studies/HPTN052Lab.htm](http://www.hptn.org/research_studies/HPTN052Lab.htm).

If you have any specific questions relating to the FBS, please contact John Polan at Gemini Bio-Products at 1-800-543-6464 ext. 302, 1-530-668-3636, or via email at [jpolan@gembio.com](mailto:jpolan@gembio.com), the customer service department at 1-800-543-6464 ext 104, or consult your affiliate U.S. institution if you are having trouble obtaining this item.

#### **10.12.4 pH Paper Ordering Information**

Allegiance-brand pH paper for the study will be provided by the HPTN Network Laboratory; please contact Estelle Piwowar-Manning at 410-614-6736 (phone), 410-614-0430 (fax) or [epiwowa@jhmi.edu](mailto:epiwowa@jhmi.edu) to arrange for the shipment of the pH paper.

#### **10.12.5 Specimen Shipping Boxes Ordering Information**

If the site has any questions regarding ordering and purchasing of the appropriate shipping boxes, please contact your local courier company. Please consult your affiliate U.S. institution if you are having trouble obtaining this item.