# Section 8. Laboratory Considerations

## 8.1 Overview and General Guidance

This section contains information on the laboratory procedures performed in MTN-009 and is intended to standardize laboratory procedures.

All laboratory procedures should be performed in accordance with the DAIDS *Requirements for DAIDS Funded and/or Sponsored Laboratories in Clinical Trials*, the HPTN-MTN Network Laboratory Manual, test kit/method package inserts (when applicable), and approved site standard operating procedures (SOPs). The DAIDS requirements can be accessed at:

http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/Laboratories.htm

The HPTN-MTN Network Laboratory manual can be accessed at:

www.mtnstopshiv.org

All site laboratories will be monitored by the MTN Network Laboratory (NL) which will utilize information from DAIDS monitoring groups (PNL, IQA, VQA, etc) to monitor and certify laboratories for testing. MTN NL approval of site laboratory proficiency is required prior to site-specific study activation.

As transmission of HIV and other infectious agents can occur through contact with contaminated needles, blood, blood products, and vaginal secretions, all study staff must take appropriate precautions when collecting and handling biological specimens. Sites must have appropriate written safety procedures in place before study initiation. Guidance on universal precautions available from the US Centers for Disease Control and Prevention can be found at the following websites:

http://www.cdc.gov/ncidod/dhqp/bp_universal_precautions.html

Some laboratory procedures will be performed in study site clinics or laboratories; others will be performed in the MTN NL in Pittsburgh, PA. Table 8-1 lists for each test the testing location, specimen type, specimen container and kit/method (if mandated).

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

Ideally, one method, type of test kit, and/or combination of test kits will be used for each protocol specified test throughout the duration of the study. Due to the duration of this study, this may not be feasible. 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. The MTN NL must be notified before the change and can provide further guidance on validation requirements. Similarly, all labs must contact the MTN NL in cases of changes to normal ranges.

**Table 8-1**
**Overview of Laboratory Testing Locations, Specimens, and Methods for MTN-009**

| Test | Testing Location | Specimen Type | Tube/ Container | Specimen Criteria | Kit/Method |
|---|---|---|---|---|---|
| HIV antibody screen | Local Lab | Plasma or Whole Blood | EDTA tube/ Finger Stick | Locally defined | Two different rapid tests; at least one must be FDA-approved |
| Western Blot | Local Lab | Plasma | EDTA Tube | Locally defined | Genetics Systems Bio-Rad FDA Approved |
| Plasma HIV-1 RNA Viral Load | Network Lab (Virology Core) | Plasma | EDTA tube | 2 ml EDTA Plasma frozen ≤ -70°C | Abbott RealTi*m*e HIV-1 *m*2000 System |
| HIV-1 Genetic Resistance Test | Network Lab (Virology core) | Plasma | EDTA tube | 5 ml EDTA Plasma frozen ≤-70°C | Genotypic characterization |
| CD4+ T Cell Count | Local Lab | Whole Blood | EDTA tube | Locally defined | Not specified |
| Categorization as recent or chronic infection | Network Lab (Virology core) | Plasma | EDTA tube | 1 ml EDTA Plasma frozen ≤-70°C | Sedia™ BED HIV-1 Incidence EIA |

Sites are responsible to ensure that specimen volumes do not exceed what is described in the informed consent process. The MTN NL may request details of collection containers and volumes for this purpose. These blood draws will vary by site.

## 8.2 Specimen Labeling

All containers into which specimens are initially collected (e.g., urine collection cups, blood collection tubes) will be labeled with SCHARP-provided Participant ID (PTID) labels. The date the specimens are collected should also be entered on the label. If the date is handwritten, it should be in indelible ink (such as a Sharpie pen). 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. The following specimens will be entered into Laboratory Data Management System (LDMS) and labeled with LDMS-generated labels, using the “LDMS1” format: plasma for storage.

See Section 8.3 for more information on use of LDMS for this study.

## 8.3 Use of LDMS

LDMS is a computer program used to track the storage and shipping of laboratory specimens. It is supported by the Frontier Science Foundation (FSTRF). LDMS must be used at all sites to track the collection, storage, and shipment of plasma that will be sent to the MTN NL for testing. LDMS shipping manifests will be used.

Detailed instructions for use of LDMS are provided at: https://www.fstrf.org/ldms (may require a password).

For MTN-009, use the LDMS1 label format.

All sites are required to maintain the current version of LDMS and monitor updates relating to use of the LDMS. It is crucial to be aware of proper label formats to ensure that specimens are correctly labeled. Sites are responsible to back up their LDMS data (frequency determined by site) locally and to export their data to FSTRF (at least weekly).

Questions related to use of LDMS in MTN-015 may be directed to Edward Livant or LDMS Technical (User) Support. Usual business hours for LDMS User Support are 7:30 am - 6:00 pm ET on Monday and Fridays and 7:30 am - 8:00 pm ET on Tuesdays, Wednesdays, and Thursdays. During business hours, please contact LDMS User Support as follows:

Email: ldmshelp@fstrf.org
Phone: +716-834-0900, ext 7311
Fax: +716-898-7711

LDMS User Support can be paged via email during non-US 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, 2 or 3 (addresses shown in Table 3 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

FSTRF no longer supports the use of pagers. The email addresses in Table 8-2 can still be used as needed.

**Table 8-2**
**LDMS User Support Paging Details**

| Pager | Email Address |
|---|---|
| LDMS 1 | ldmspager1@fstrf.org |
| LDMS 2 | ldmspager2@fstrf.org |
| LDMS 3 | ldmspager3@fstrf.org |

Each site must export its LDMS data to FSTRF on a weekly basis. Exported data are used by the MTN Statistical and Data Management Center (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 MTN 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 MTN 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 MTN NL and SDMC will discuss and document any items that, although resolved, appear 'irresolvable' in LDMS.

Table 8-3 should be used as a guide when logging MTN-009 specimens into LDMS. Please use the LDMS codes listed in the table when logging in specimens for each test listed.

**Table 8-3**
**LDMS Specimen Management Guide to Logging in MTN-009 Specimens**

| Test | Primary | Primary Additive | Primary Volume | Primary Units | Aliquot Derivative | Aliquot Sub Add/Der | Aliquot Volume | Aliquot Units |
|---|---|---|---|---|---|---|---|---|
| Plasma for storage | BLD | EDT | Variable | ml | PL 1/2 | N/A | 0.5 | N/A |

Section Appendix 8-3 shows the LDMS tracking sheet to be used.

## 8.4 Blood Collection, Processing, Testing, and Storage

The blood tests performed at each study visit vary depending on the time point of the visit and potentially the clinical presentation of the participant. Perform all tests according to site SOPs and package inserts.

### 8.4.1 Specimen Collection and Initial Processing

Label all required tubes with a SCHARP-provided PTID label at the time of collection. After collection, EDTA tubes should be gently inverted at least eight times after specimen collection to prevent clotting. If whole blood is to be used for multiple tests, ensure that the tube is well mixed before removing any specimen.

All enrolled participants will initially have a fingerstick for HIV rapid tests. If the either or both of the results are positive, a venous sample will be drawn into EDTA vacutainers.

### 8.4.2 HIV-1 RNA Viral Load

HIV RNA viral load will be performed in the MTN Virology Core Laboratory from EDTA plasma using the Abbott RealTi*m*e HIV-1 *m*2000 System. This assay is real-time PCR-based, which has a lower limit of quantification of 40 copies/ml. All testing will be performed according to Pittsburgh Virology Core SOPs and package inserts.

Follow plasma archive specimen requirements in Section 8.4.6

### 8.4.3 HIV-1 Genotypic Resistance

HIV-1 standard genotypic resistance testing will take place at the MTN Virology Core Laboratory using the ViroSeq™ HIV-1 Genotyping System.

HIV-1 sensitive genotypic resistance testing will take place at the MTN Virology Core Laboratory. An in-house, allele-specific PCR assay will be used to detect the percent frequency of the HIV-1 reverse transcriptase mutations K65R, K103N, Y181C and M184V/I in HIV-1 from plasma samples of HIV-positive MTN-009 participants.

The preferred sample is five 1 ml aliquots; the minimum sample is four 1 ml aliquots. The specimens are stored at ≤ -70°C.

Follow plasma archive specimen requirements in Section 8.4.6

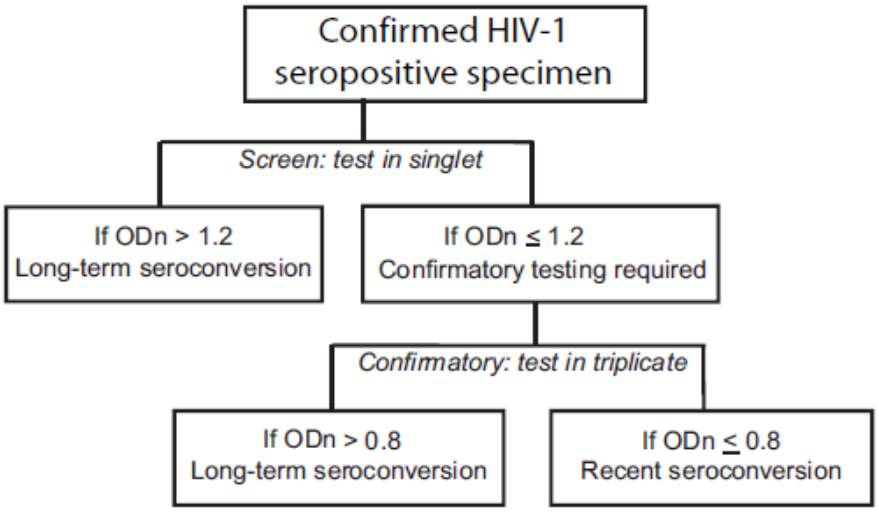
### 8.4.4 Recent/Chronic Infection

The Sedia™ BED HIV-1 Incidence Enzyme Immunoassay (EIA) will be performed in the MTN Virology Core Laboratory from EDTA plasma. The Sedia™ assay is an *in vitro* quantitative EIA for distinguishing recent HIV-1 infections from those which are long-term. The test measures the proportion of HIV-1 specific IgG to total IgG in blood samples including plasma. This assay is for research purposes only and is not intended for diagnostic procedures or for determining clinical outcome or treatment. Results from this assay cannot be given to participants for clinical management.

The BED EIA should only be done on confirmed HIV-1 seropositive specimens. Each specimen will be tested in singlet. Samples with $OD_{450}$ values > 1.2 will be classified as long-term HIV infections. Samples with $OD_{450} \leq 1.2$ will be repeated in triplicate. $OD_{450}$ during confirmatory testing must be >0.8 to be classified as long-term infection (Figure 8-1).

Follow plasma archive specimen requirements in Section 8.4.6

**Figure 8-1**
**Algorithm for Interpreting Sedia™ BED EIA Results**




### 8.4.5 CD4+ T Cell Count

Site laboratories will test EDTA whole blood by flow cytometry for absolute CD4+ T Cell counts per local SOPs. Testing will be performed on FDA approved instruments per site SOPs and package inserts. Sites must participate in United Kingdom External Quality Assurance (UKNEQAS) programs and be approved by the Immunology Quality Assurance (IQA) group to perform this testing.

### 8.4.6 Plasma Archive

Plasma will be processed and stored at all visits. Plasma will be stored at ≤ -70°C and logged into LDMS. If held at room temperature, plasma must be frozen within 4 hours of collection. If refrigerated or on ice, plasma must be frozen within 24 hours of collection.

At visits when plasma archive is required, store all available plasma in 1 mL aliquots. The desired amount of plasma per required visit is at least 8 mL of plasma with a minimum of 6 ml. Notify the MTN NL of any cases of less then 6 mL being stored.

### 8.4.7 Rapid HIV and Western Blot Testing

Plasma or whole blood will be tested for HIV using tests that have been validated at the study site. At all sites, HIV infection status will be assessed per the testing algorithm in protocol Appendices II; these algorithms are also provided in appendix 8-1.

Sites will use two rapid HIV tests at screening. At least one of the rapid tests must be FDA approved. A second qualified staff member must read and verify results within allowable time frame for the tests.

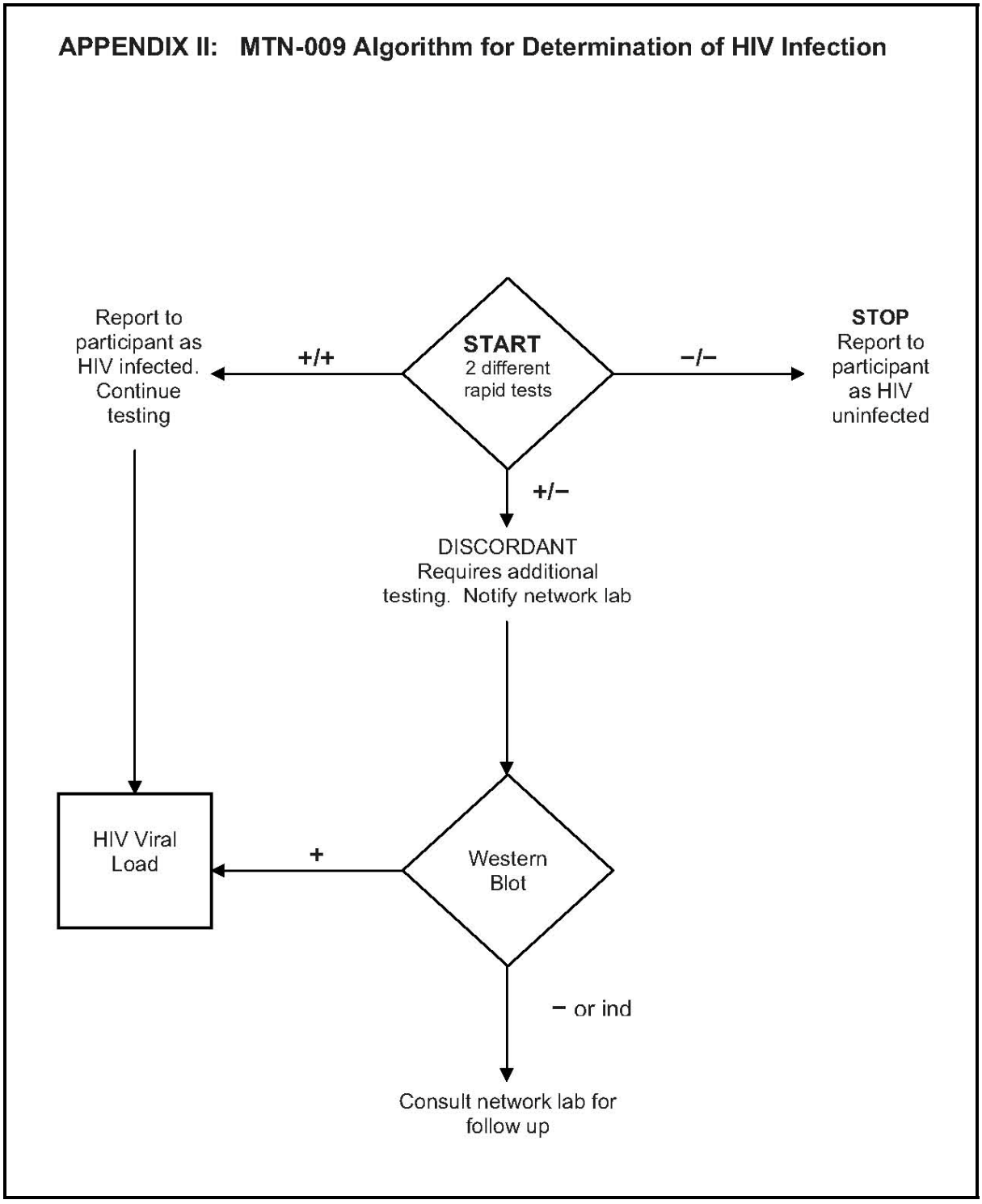
If both rapids are negative, the participant will be considered HIV-uninfected. If both are positive, the participant will be considered HIV-infected.

If the rapid tests are discordant, i.e., one rapid test is positive and one is negative, the Genetic Systems FDA-approved WB will be performed. Inform the MTN NL whenever discordant results are obtained. The NL may provide technical guidance at this time if needed; however WB testing at the local lab should proceed immediately upon identification of at least one discordant rapid tests.

If the WB is negative, the participant will be considered HIV-uninfected. If the WB is positive, the participant will be considered HIV-infected. If the WB is indeterminate, consult the NL for guidance.

All participants who have 2 positive rapid tests or a positive Western Blot will have plasma stored for testing at the NL and a CD4 count done locally.

## Section Appendix 8-1 MTN 009 HIV Algorithm for Determination of HIV Infection



## Section Appendix 8-2 MTN 009 HIV Algorithm for Determination of HIV Infection

**From Bennett et al. (2009) Drug Resistance Mutations for Surveillance of Transmitted HIV-1 Drug-Resistance: 2009 Update PLoS One. 4(3): e4724.**

### Nucleoside RT Inhbitor Surveillance Drug Resistance Mutations

**Table 2.** Nucleoside RT Inhibitor Surveillance Drug Resistance Mutation (SDRM) List: 34 Mutations at 15 Positions

| Position | AA | Lists | New | A (%) | AE (%) | AG (%) | B (%) | C (%) | D (%) | F (%) | G (%) | No Rx (Max %) | Max Rx (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Number of individuals:* | | | | 1,305 | 770 | 1,035 | 5,672 | 2,020 | 324 | 265 | 403 | 11,586 | 14,621 |
| 41 | L | 5 | | 0 | 0.3 | 0.1 | 0.3 | 0 | **1.2** | 0 | 0 | 1.2 | 39 |
| 65 | R | 5 | | 0.1 | 0 | 0 | 0 | 0.2 | 0 | 0 | 0 | 0.2 | 6.5 |
| 67 | N | 5 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 44 |
| | G | 4 | | 0 | 0 | 0.1 | 0.2 | 0.1 | 0 | 0 | 0 | 0.2 | 1.6 |
| | E | 3 | √ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.7 |
| 69 | D | 4 | | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 7.1 |
| | ins | 5 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.2 |
| 70 | R | 5 | | 0 | 0.3 | 0 | 0.2 | 0 | 0 | 0 | 0 | 0.3 | 29 |
| | E | 5 | √ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.6 |
| 74 | V | 5 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 | 0.2 | 7.5 |
| | I | 4 | √ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.3 |
| 75 | M | 4 | | 0 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 3.8 |
| | T | 4 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.7 |
| | A | 3 | | 0 | 0 | 0 | 0 | 0.1 | 0 | 0 | 0 | 0.1 | 0.8 |
| | S | 3 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.4 |
| 77 | L | 3 | | 0 | 0.3 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0.3 | 2.0 |
| 115 | F | 5 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.4 |
| 116 | Y | 3 | | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 3.3 |
| 151 | M | 5 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5.2 |
| 184 | V | 5 | | 0.2 | 0.1 | 0.2 | 0.2 | 0.1 | **0.6** | 0 | 0 | 0.6 | 61 |
| | I | 5 | | 0 | 0.3 | 0.1 | 0 | 0 | 0.3 | 0 | 0 | 0.3 | 2.8 |
| 210 | W | 5 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 |
| 215 | Y | 5 | | 0.2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 | 38 |
| | F | 5 | | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 22 |
| | I | 3 | | 0.2 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 | 5.7 |
| | S | 3 | | 0 | 0.1 | 0.1 | 0.4 | 0 | 0 | 0.4 | 0 | 0.4 | 0.6 |
| | C | 3 | | 0 | 0 | 0 | 0.2 | 0 | 0 | 0 | 0 | 0.2 | 3.1 |
| | D | 3 | | 0 | 0 | 0 | 0.4 | 0 | 0.3 | 0 | 0 | 0.4 | 0.8 |
| | V | 3 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.0 |
| | E | 3 | | 0 | 0 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0.1 | 2.1 |
| 219 | Q | 5 | | 0.2 | 0.4 | 0.4 | 0 | 0 | 0 | 0 | 0 | 0.4 | 25 |
| | E | 5 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9.4 |
| | N | 4 | √ | 0.1 | 0.4 | 0.2 | 0.1 | 0 | 0 | 0 | 0 | 0.4 | 2.5 |
| | R | 4 | | 0.2 | 0 | 0.1 | 0.1 | 0.1 | 0 | 0 | 0 | 0.1 | 2.2 |
| *Sum of Prevalences:* | | | | **1.4** | **2.9** | **1.3** | **2.3** | **0.6** | **2.4** | **0.4** | **0.2** | | |

**Abbreviations:** Pos – amino acid position; AA – amino acid difference from consensus B; Lists – Number of mutation lists with the mutation; New – mutations not present on the 2007 SDRM list; No Rx – highest prevalence in untreated persons in any of the 8 listed subtypes; Max Rx – Prevalence of the mutation in the subtype with the highest prevalence of the mutation provided the mutation is present in viruses from two or more individuals. Undefined bold mutations are those with a prevalence >0.5% that are nonetheless included because the >0.5% prevalence is in only one subtype with fewer than 1000 sequences available for analysis.
doi:10.1371/journal.pone.0004724.t002

## Non-Nucleoside RT Inhibitor Surveillance Drug Resistance Mutations

**Table 3.** Non-Nucleoside RT Inhibitor Surveillance Drug Resistance Mutation (SDRM) List: 19 Mutations at 10 Positions

| Position | AA | Lists | New | A (%) | AE (%) | AG (%) | B (%) | C (%) | D (%) | F (%) | G (%) | No Rx (Max %) | Max Rx (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Number of individuals:* | | | | **1,305** | **770** | **1,035** | **5,672** | **2,020** | **324** | **265** | **403** | **11,784** | **14,621** |
| 100 | I | 5 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5.4 |
| 101 | E | 5 | | 0 | 0 | 0 | 0.2 | 0 | 0 | 0 | 0 | 0.2 | 6.4 |
| | P | 5 | √ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.4 |
| 103 | N | 5 | | 0.1 | 0 | 0.2 | 0.3 | 0.2 | 0 | 0 | 0 | 0.3 | 40 |
| | S | 4 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.6 |
| 106 | M | 5 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 |
| | A | 5 | | 0 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0.1 | 1.1 |
| 179 | F | 5 | √ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 |
| 181 | C | 5 | | 0.1 | 0.1 | 0.1 | 0 | 0.1 | 0 | 0 | 0 | 0.1 | 14 |
| | I | 5 | | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 1.1 |
| | V | 5 | √ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.1 |
| 188 | L | 5 | | 0 | 0.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0.3 | 2.4 |
| | H | 5 | | 0 | 0 | 0 | 0.1 | 0 | 0.3 | 0 | 0 | 0.3 | 0.6 |
| | C | 5 | | 0 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0.9 |
| 190 | A | 5 | | 0.1 | 0 | 0 | 0 | 0.1 | 0.3 | 0 | 0 | 0.3 | 10 |
| | S | 5 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.6 |
| | E | 4 | | 0 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0.5 |
| 225 | H | 5 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7.6 |
| 230 | L | 3 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.6 |
| *Sum of Prevalences:* | | | | **0.4** | **0.4** | **0.6** | **0.7** | **0.5** | **0.6** | **0** | **0** | | |

**Abbreviations:** Pos – amino acid position; AA – amino acid difference from consensus B; Lists – Number of mutation lists with the mutation; New – mutations not present on the 2007 SDRM list; No Rx – highest prevalence in untreated persons in any of the 8 listed subtypes; Max Rx – Prevalence of the mutation in the subtype with the highest prevalence of the mutation provided the mutation is present in viruses from two or more individuals.

doi:10.1371/journal.pone.0004724.t003

## Protease Inhibitor Surveillance Drug Resistance Mutations

**Table 4.** Protease Inhibitor (PI) Surveillance Drug Resistance Mutation (SDRM) List: 40 Mutations at 18 Positions*

| Position | AA | New | Lists | A (%) | AE (%) | AG (%) | B (%) | C (%) | D (%) | F (%) | G (%) | No Rx (%) | Max Rx (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Number of individuals:* | | | | 1,528 | 902 | 1,437 | 7,439 | 2,182 | 515 | 598 | 619 | 15,220 | 7,886 |
| 23 | I | √ | 3 | 0.1 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 2.1 |
| 24 | I | | 5 | 0 | 0 | 0.1 | 0 | 0 | 0 | 0.2 | 0 | 0.2 | 11 |
| 30 | N | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9.0 |
| 32 | I | | 5 | 0 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0.1 | 7.3 |
| 46 | I | | 5 | 0.3 | **0.6** | 0.1 | 0.4 | 0.2 | 0 | 0.2 | 0 | 0.6 | 29 |
| | L | √ | 5 | 0.3 | 0.2 | 0.3 | 0.3 | 0.1 | 0.2 | 0.2 | 0.5 | 0.5 | 13 |
| 47 | V | | 5 | 0 | 0 | 0 | 0 | 0.1 | 0.2 | 0 | 0.3 | 0.3 | 6.8 |
| | A | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 |
| 48 | V | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5.7 |
| | M | √ | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.3 |
| 50 | V | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.5 |
| | L | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.8 |
| 53 | L | | 5 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0.2 | 0.2 | 7.8 |
| | Y | √ | 4 | 0.1 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0.6 |
| 54 | V | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 43 |
| | L | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4.6 |
| | M | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4.3 |
| | A | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.6 |
| | T | | 5 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 1.7 |
| | S | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.7 |
| 73 | S | | 5 | 0 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0.1 | 13 |
| | T | | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4.3 |
| | C | | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.0 |
| | A | | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.8 |
| 76 | V | √ | 5 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0 | 0 | 0.1 | 4.3 |
| 82 | A | | 5 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0.2 | 0 | 0.2 | 30 |
| | T | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 |
| | F | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.1 |
| | S | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.6 |
| | C | √ | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.8 |
| | M | | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.5 |
| | L | √ | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 |
| 83 | D | √ | 3 | 0 | 0 | 0.1 | 0 | 0.1 | 0 | 0 | 0 | 0.1 | 9.3 |
| 84 | V | | 5 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 21 |
| | A | | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.8 |
| | C | | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.8 |
| 85 | V | √ | 4 | 0.1 | 0 | 0.1 | 0.1 | 0.2 | 0 | 0.2 | 0 | 0.2 | 6.4 |
| 88 | D | | 5 | 0 | 0.1 | 0.1 | 0.1 | 0 | 0 | 0 | 0.2 | 0.2 | 6.4 |
| | S | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.4 |
| 90 | M | | 5 | 0.1 | 0.3 | 0 | 0.1 | 0.1 | 0 | 0 | 0.5 | 0.5 | 45 |
| *Sum of Prevalences:* | | | | 1.1 | 1.5 | 1.1 | 1.0 | 1.0 | 0.4 | 1.2 | 1.2 | | |

**Abbreviations:** Pos – amino acid position; AA – amino acid difference from consensus B; Lists – Number of mutation lists with the mutation; New – mutations not present on the 2007 SDRM list; No Rx – highest prevalence in untreated persons in any of the 8 listed subtypes; Max Rx – Prevalence of the mutation in the subtype with the highest prevalence of the mutation provided the mutation is present in viruses from two or more individuals. Underlined bold mutations are those with a prevalence >0.5% that are nonetheless included because the >0.5% prevalence occurs in only one subtype with fewer than 1000 sequences or in fewer than 1000 sequences available for analysis.

doi:10.1371/journal.pone.0004724.t004

**Section Appendix 8-3 LDMS Tracking Sheet**

# MTN 009 Non-DataFax LDMS Specimen Tracking Sheet

*For log-in of MTN 009 stored specimens into LDMS*

**Participant ID** ☐☐☐-☐☐☐☐☐-☐ (Site Number – Participant Number – Chk)

**Visit Code** ☐☐.☐

**Specimen Collection Date** ☐☐ ☐☐☐ ☐☐ (*dd MMM yy*)

| # of TUBES | PRIMARY SPECIMEN | PRIMARY ADDITIVE | ALIQUOT DERIVATIVE | ALIQUOT SUB ADDITIVE/ DERIVATIVE | NOTES FOR LAB |
|---|---|---|---|---|---|
| ☐ | Blood **(BLD)** *Plasma* | **EDT** (purple top) | **PL 1/2** | **N/A** | Store in aliquots of 1-2 ml. If held at room temperature, plasma must be frozen within 4 hours of collection. If refrigerated or on ice, plasma must be frozen within 24 hours of collection. Plasma can be single or double spun. |

Comments: _____

**Initials:** _____ (Sending Staff) _____ (Receiving Staff) **LDMS Data Entry Date:** ☐☐ ☐☐☐ ☐☐ (*dd MMM yy*) / _____ (LDMS Staff)

**Final Version 1.0, 18-MAR-10**