Section 12. Laboratory Considerations

12.1 Overview and General Guidance

This section contains information on the laboratory procedures performed in MTN-016, which are limited to testing on HIV-exposed and/or HIV-infected infants. The tests to be conducted on these infants include: HIV-1 DNA PCR, HIV-1 RNA PCR (confirmatory testing) and standard genotypic resistance testing; plasma will be processed for storage and later testing.

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 website:

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

Some laboratory procedures will be performed in the study site clinic or laboratory and others in the MTN Network Laboratory (NL).

Table 12-1 lists for each test the testing location, specimen type, specimen container and kit/method (if specified). Table 12-2 specifies blood collection by visit type and suggested volumes.

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

Questions related to MTN-016 laboratory procedures may be referred to:

Pam Kunjara Magee-Womens Research Institute 204 Craft Ave, Room A540 Pittsburgh, PA 15213 rsipk@mwri.magee.edu Phone # 412-641-6393 Pager # 412-917-9343 Fax # 412-641-6170	Urvi Parikh University of Pittsburgh 3550 Terrace Street Scaife Hall, Suite 817-A Pittsburgh, PA 15261 ump3@pitt.edu Phone # 412-648-3103 Fax # 412-648-8521

Table 12-1
Overview of Laboratory Testing Locations, Specimens, and Methods for MTN-016

Test	Testing Location	Specimen Type	Tube/Container	Kit/Method
HIV-1 DNA	Local or Regional	Whole Blood cell pellet or Dried Blood Spots	EDTA tube	Roche Amplicor HIV-1 DNA Test, COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, Abbott RealTime HIV-1 Qualitative or Network approved method
HIV-1 RNA	Local, Regional, or Network Lab	Plasma	EDTA tube	Roche Amplicor HIV-1 Monitor Test, AmpliPrep/COBAS® TaqMan® HIV-1, Abbott RealTime HIV-1 assay or Network approved method
Genotypic Resistance testing	MTN NL	Plasma	EDTA tube	ViroSeq or Network approved method

Table 12-2
Suggested Volumes for Infant Blood Collection

Visit Type	Total Blood Volume (ml)*	Purpose
Initial HIV testing with confirmation	EDTA tube: 2 ml	HIV DNA, HIV RNA
Resistance Testing	EDTA tube: 3ml**	Resistance testing
Interim Visit	Varies	As clinically indicated

** Volumes may vary depending on each site's testing platforms. Please confirm with the testing lab to determine minimum volume requirements. Sites are responsible for ensuring 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.*
***A minimum of 1 ml plasma must be available for resistance testing to be done.*

Ideally, one method, one type of test kit, and/or a 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 test kit must be used after study initiation, site laboratory staff must perform a validation study of the new method or test prior to implementing a change in methods. The MTN NL must be notified before implementing the change and the MTN NL can provide further guidance on validation requirements.

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 all regulatory authorities.

This section of the MTN-016 SSP manual gives basic guidance to the sites but is not an exhaustive procedure manual for all laboratory testing. This section must be supplemented with Standard Operating Procedures. The MTN NL will assist in the creation of any SOPs upon request. Essential SOPs to be created by the site for MTN-016 include but are not limited to:

- Specimen Collection and transport
- Chain of Custody (Must be approved by the MTN NL for study activation)
- HIV testing

12.2 Specimen Labeling

All containers into which specimens are initially collected (e.g. blood collection tubes) will be labeled with SCHARP-provided Participant ID (PTID) labels. The date of specimen collection should also be included on the label. If the date is handwritten, it should be in indelible ink (such as a Sharpie pen).

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 LDMS and labeled with LDMS-generated labels: HIV DNA PCR, HIV RNA PCR, HIV Resistance testing.

12.3 Procedures for Specimens that cannot be evaluated

When possible, specimens will be redrawn or recollected if it is found that they cannot be evaluated per site SOPs. The site will monitor specimen management problems as part of ongoing Quality Assurance. In cases where additional specimens need to be recollected due to a laboratory error (lost or broken specimen or clerical error) or a clinic error (clerical error), a protocol event form provided by the NL may be required.

12.4 Use of LDMS

The Laboratory Data and Management System (LDMS) is a program used for the storage and shipping of laboratory specimens. It is supported by the Frontier Science Foundation (FSTRF). LDMS must be used to track the collection, storage, and shipment of all specimens collected for MTN-016.

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

The site will be 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. The site will be 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-016 may be directed to Pam Kunjara or Urvi Parikh at the NL or LDMS Technical (User) Support. Usual business hours for LDMS User Support are 7:00 am - 6:00 pm (U.S. ET) on Monday through Friday. All other hours and weekends, an on-call user support specialist will be available. Contact LDMS User Support at:

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

Off-Hours Contact Information
If you are locked out of your LDMS or are experiencing errors that prevent you from completing your LDMS lab work during off-hours, page LDMS User Support using the LDMS Web Pager utility. Alternatively, you may e-mail the paging system directly at ldmspager1@fstrf.org. Please allow at least 15 minutes to get a response before sending another e-mail to the paging system.

Each site must export its LDMS data to Frontier Science (FSTRF) on a weekly basis. Exported data are used by the MTN 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 the 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 (e.g., blood needed for confirmatory HIV testing) 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 'irresolvable' in LDMS.

Table 12-4
LDMS Specimen Management Guide to Logging in 016 Specimens

The table below should be used as a guide when logging in MTN 016 specimens. Please use the LDMS codes listed below when logging in specimens for each test listed. Tests that are listed as local do not require that a sample be logged into the LDMS. See Appendix 12-1 for a copy of the LDMS tracking sheet.

Test	Primary	Additive	Derivative	Primary Volume	Aliquot Volume	Units
*Blood for HIV DNA and RNA PCR testing	BLD	EDT	PL1/2	2ml***	Minimum of 0.5***	ML
		EDT	PER	2ml	5 x 10^6	CEL
		EDT	DBS	2 ml	100	UL
**Blood for HIV resistance testing	BLD	EDT	PL1/2	Please contact Virology Core	1	ML

** For determination of infant HIV status DNA and RNA PCR are performed from same specimen. RNA PCR is performed only when DNA PCR is positive.*
*** RNA PCR may be performed for resistance analysis if viral load is not already available*
**** Volumes may vary depending on each site's testing platforms. Please confirm with the testing lab to determine minimum volume requirements.*

Table 12-5
Specimen Shipping Summary

Specimen	Use LDMS?	Ship to:	Shipping schedule
Blood for DNA/RNA PCR	Yes	If not performed locally –Regional or MTN Network Lab - Pittsburgh	Within 1 week of processing
Plasma for resistance testing	Yes	MTN Core Virology – Pittsburgh	Contact MTN Core Virology for shipping schedule

12.5 HIV Testing for Infants

If the woman is known to be HIV positive, she has the option to test her infant for HIV status and possible resistance, for up to 6 weeks from cessation of breast feeding. Maternal diagnosis will ideally be based upon documented HIV testing through the parent protocol. However, because some women may be diagnosed outside of any related clinical trial, MTN-016 staff may presume that an infant is HIV-exposed based on maternal report alone.

All tests, and associated procedures, must be documented on local laboratory log sheets or other laboratory source documents. At all sites, HIV infection status will be assessed or confirmed per the MTN-016 HIV testing algorithm (see appendix 12-2 and 12-3 in this section of the MTN-016 SSP).

12.5.1 Specimen Collection and Initial Processing

Label all required tubes with a SCHARP-provided PTID label at the time of collection.
After collection, lavender top tubes (additive = EDTA) should be gently inverted at least eight times to prevent clotting. EDTA tubes are used for all HIV-1 testing in infants and must be processed within 6 hours of collection.

Note: If locally available tube top colors do not correspond with the tube additives specified above, use appropriate tubes based on the additives, not the listed tube top colors.

12.5.2 HIV-I DNA PCR and RNA PCR Testing

These tests can be performed locally or regionally.

For local and regional testing, please follow site SOPs and standard of care. Before site activation, the MTN NL will review and approve testing platforms to be used at the site level. Sites that perform testing locally for MTN-016 will participate in the Virology Quality Assurance (VQA) External Quality Assurance program. Any changes to testing platforms such as using Dried Blood Spots (DBS) instead of cell pellets for DNA PCR must be approved by the MTN NL in accordance with VQA validation standards. All tests and associated QC procedures must be documented on local laboratory log sheets or other laboratory source documents.

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). Notify the NL

immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken.

At all sites, all test results must be documented on local laboratory log sheets or other laboratory source documents. In addition to initialing or signing the testing logs to document review and verification of the results, the second staff member must also record the time at which the results were reviewed and verified.

Steps to follow once an infant has been identified as HIV-1 exposed (Appendix 12-2)
Sample 1: 2ml of whole blood (EDTA) will be needed to perform HIV-1 testing in infants by HIV-1 DNA PCR and RNA PCR [viral load]. From the 2ml whole blood (EDTA) 1cell pellet [5×10^6 cell] for HIV-1 DNA PCR and 1ml plasma for HIV-1 RNA are required for confirmation.

The results will be interpreted as follows:

- If the HIV-1 DNA PCR test is negative for Sample 1, the infant is considered HIV-1 uninfected and may need to be retested at a later date if there is continued exposure to HIV-1 through breast feeding.
- If the HIV-1 DNA PCR test is positive for Sample 1, the quantitative standard HIV-1 RNA PCR assay which determines viral load will be performed.
- If the viral load is below the limit of detection, please contact the NL for further analysis and instructions.
- If the viral load is detectable a second sample (Sample 2) will be collected at the interim visit.

Sample 2: At the interim visit collect 2ml of whole blood (EDTA) for a repeat HIV-1 RNA PCR and 3ml of whole blood (EDTA) for resistance testing.

- If the viral load is below the limit of detection, please contact the NL for further analysis and instructions.
- If the viral load is detectable, the plasma sample will be referred for resistance testing.
- The infant is only considered HIV-1 infected after 2 HIV-1 RNA PCR specimens are confirmed to have detectable viral loads.

Steps to follow once infant is considered HIV-1 infected (Appendix 12-3)
Once an infant has been confirmed HIV-1 infected at each follow up visit collect 2ml of whole blood (EDTA) for HIV-1 RNA PCR and 3ml of whole blood EDTA for resistance testing.

12.5.3 HIV Resistance Testing

Once an infant is diagnosed as being HIV-1 infected, resistance testing may be offered. This test will be performed by the MTN Core Virology Lab using the ViroSeq assay. Based on the viral load results from the RNA PCR the volume of plasma required may vary. A minimum of 1ml plasma must be available. Therefore, we require at least 2-3ml whole blood (EDTA) to yield a minimum 1ml plasma volume.

Specimen Processing Instructions

1. Collect at least 3ml of whole blood into an EDTA microtainer by heel stick or venipuncture.
2. Specimen must be stored at 2-25°C and processed within **2** hours from collection.
3. Spin whole blood at 800-1600 ×g for 20 minutes at room temperature.
4. Separate plasma and aliquot into 1.0ml volumes per cryovial. All remaining volumes should be stored and noted in LDMS.
5. Store plasma at -70°C until ready to ship to MTN NL.

6. LDMS will be used to label and track the specimens.

12.5.4 Shipping to the MTN Core Virology Lab

HIV Infant testing (DNA PCR and RNA PCR) not being performed locally must be shipped to the regional or MTN Core Virology Lab. For DNA PCR and RNA PCR specimens going to the MTN Core Virology Lab, please ship them to Urvi Parikh (Pittsburgh). All resistance testing must be performed by the MTN Core Virology Lab and will be shipped to Urvi Parikh (Pittsburgh).

- When the site is ready to ship to the MTN Core Virology Lab please follow all local and IATA regulations for shipping diagnostic specimens.
- Use LDMS to create a shipping batch under the shipping module. Please refer to the LDMS Manual for instructions.
- Notify the MTN Core Virology Lab via e-mail with the shipment airway bill, expected shipping dates, and the attached LDMS batch file.
- Ship batch to LDMS site 470 – MTN Core Virology Lab University of Pittsburgh:

  Urvi Parikh/Krista Eskay
  University of Pittsburgh
  3550 Terrace Street
  S804 Scaife Hall
  Pittsburgh, PA 15261
  ump3@pitt.edu
  Phone # 412-648-3103
  Fax # 412-648-8521

Appendix 12-1
LDMS Tracking Sheet

MTN-016 Non-DataFax LDMS Specimen Tracking Sheet

For login of MTN-016 Infant stored specimens into LDMS

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

Visit Code ☐.☐

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

# of TUBES or SPECIMENS	PRIMARY SPECIMEN	PRIMARY ADDITIVE	# of ALIQUOT TUBES or SPECIMENS	ALIQUOT DERIVATIVE	NOTES FOR LAB
	Blood **(BLD)** *for DNA/RNA* **Collection Time:** __ __: __ __ *hour : min*	EDT (purple top)		Plasma **(PL 1/2)**	Plasma must be frozen within 6 hours of collection.
				Cell Pellet **(PER)**	Store at -70C until testing
				Dried Blood Spot **(DBS)**	Store at RT
	Blood **(BLD)** *for resistance* **Collection Time:** __ __: __ __ *hour : min*	EDT (purple top)		Plasma **(PL 1/2)**	Store in aliquots of at least 1.0 ml. Plasma must be frozen within 2 hours of collection.

Comments: _____

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

Version 3.1, 08-Aug-12

MTN-016 Non-DataFax LDMS Specimen Tracking Sheet

For login of MTN-016 Infant stored specimens into LDMS

LDMS Specimen Tracking Sheet (nonDataFax)

Purpose: This non-DataFax form is used to document collection and entry of MTN 016 specimens into the Laboratory Data Management System (LDMS).

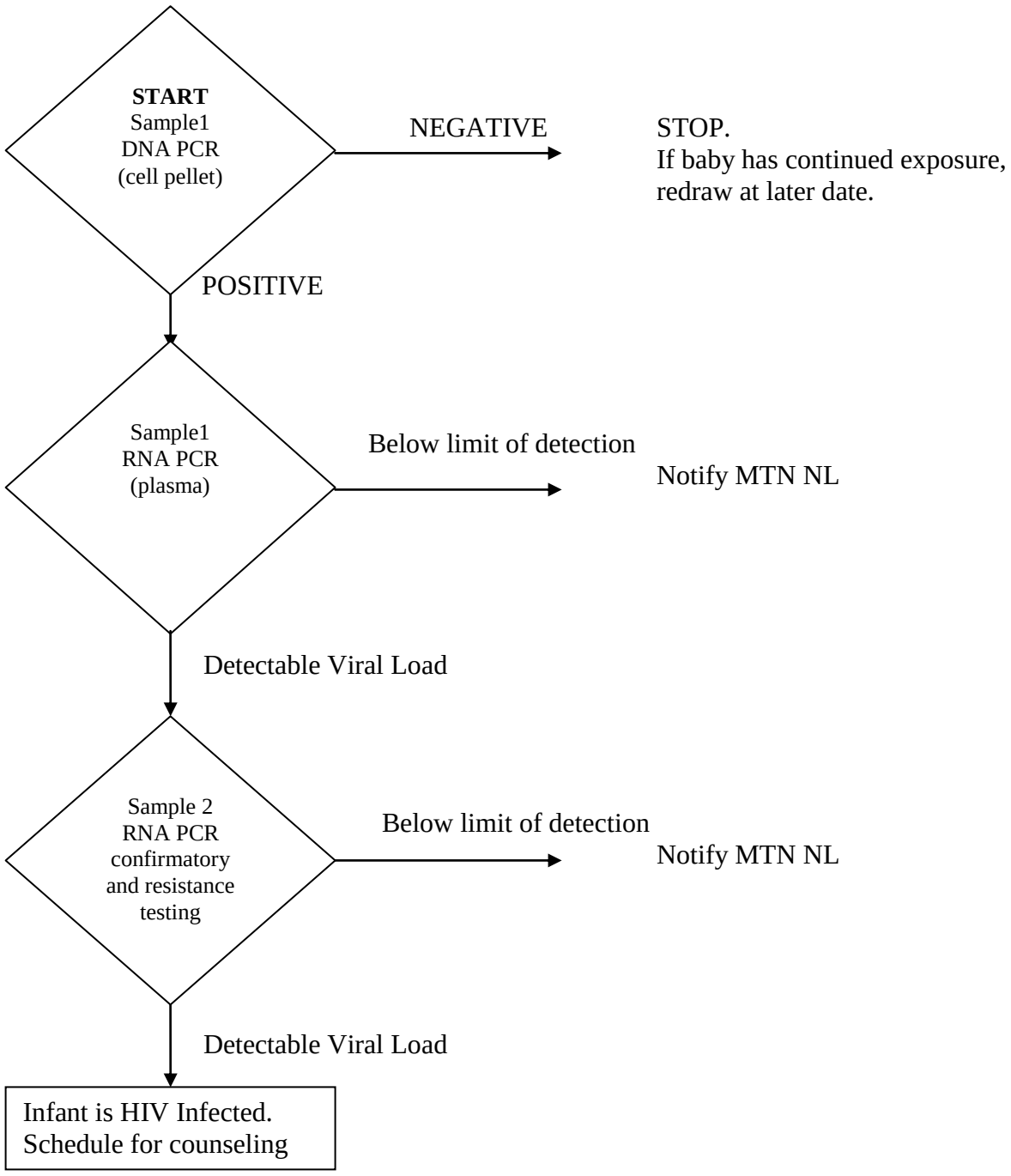
General Information/Instructions: A copy of this form accompanies LDMS specimens in their original specimen collection containers to each LDMS entry laboratory. Once the specimens have been entered into LDMS, this form is kept on file at the LDMS entry laboratory. If the site chooses, a copy of this completed form may be made once the specimens have been entered into LDMS and the copy kept in the participant's study notebook. This is not required, however. Because this form is a non-DataFax form, this form should NOT be faxed to SCHARP DataFax.

Item-specific Instructions:

- **Visit Code:** Record the visit code of the visit at which the LMDS specimens were collected.
- **# of TUBES or SPECIMENS:** Record the total number of collected tubes or specimens of the listed primary specimen type that will be entered into LDMS. If no LDMS specimens of the primary specimen type were collected, record "0."
- **Collection Time:** When collection time is present, record the time the specimen was collected using a 24-hour clock. For example, a specimen collected at 2:36pm would have "14:36" recorded as the collection time.
- **Initials – Sending Staff:** The clinic staff person who completed the form and/or who is sending the LDMS form and specimens to the LDMS entry lab, records his/her initials here.
- **Initials - Receiving Staff:** The laboratory staff person who received this form (and the LDMS specimens accompanying the form), records his/her initials here.
- **LDMS Data Entry Date:** Record the date the LDMS specimens listed on this form were entered into LDMS.
- **LDMS Data Entry Date - LDMS Staff:** The LDMS laboratory staff person who entered the specimens into LDMS, records his/her initials here.

Version 3.0, 08-DEC-11

Appendix 12-2
Algorithm for Infant HIV Testing



Appendix 12-3
Flow Chart for Testing HIV Exposed Infant within MTN-016