MTN 017 Laboratory Training

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Objectives

- Overview of Enrollment Lab testing
- HIV Confirmatory Results CRF
- Specimen Storage CRF
- Q&A

Overview of Lab Testing by Visit

	VST 1 SCR	VST 2 ENR	VST 3 MID	VST 4 END	VST 5 PD2	VST 6 MID	VST 7 END	VST 8 PD3	VST 9 MID	VST 10 END
UA	X	★	★	★	★	★	★	★	★	★
Urine GC/CT	X	★	★	★	★	★	★	★	★	X
CBC/diff/plt	X	★	★	★	★	★	★	★	★	X
AST/ALT	X	★	★	★	★	★	★	★	★	X
Creatinine	X	★	★	X	★	★	X	★	★	X
HIV serology	X	X	X	X	★	X	X	★	X	X
Plasma archive/storage		X		X			X			X
HBsAg and Ab	X									
Hep C Ab	X									
HSV serology	X									
Syphilis	X	★	★	★	★	★	★	★	★	★
Blood for PK (PBMC and plasma)			X (plasma only)	X		X (plasma only)	X		X (plasma only)	X
Coagulation (PT/INR)*	X									

Overview of Lab Testing by Visit

	VST 1 SCR	VST 2 ENR	VST 3 MID	VST 4 END	VST 5 PD2	VST 6 MID	VST 7 END	VST 8 PD3	VST 9 MID	VST 10 END
Rectal HSV detection	★	★	★	★	★	★	★	★	★	★
Anal HPV		X								
Rectal GC/CT	X	X	★	X	★	★	X	★	★	X
Rectal sponge for PK			X	X	X	X	X	X	X	X
Rectal sponge for PD		X	X	X	X	X	X	X	X	X
Rectal sponge immuno*		X		X			X			X
Rectal biopsy Proteomics*		X		X			X			X
Rectal biopsy Histology*		X		X			X			X
Rectal biopsies Pheno*		X		X			X			X
Rectal biopsy Gene Array*		X		X			X			X
Rectal biopsies PD*		X		X			X			X
Rectal biopsies PK*				X			X			X

*Tissue subset only

Overview of Lab Testing
Blood Specimens

- Plasma Archive (baseline) / Plasma Storage
  - Plasma archive is collected at enrollment
  - Plasma storage is collected at follow-up when indicated and in the event of a positive HIV rapid test after enrollment.
  - Freeze plasma within 4 hours if held at RT. If refrigerated or on ice, freeze within 24 hours.

Overview of Lab Testing

Rectal Specimens

- Swabs
  - NAAT for GC/CT (avoid gel)
  - HSV (if indicated)
  - HPV (enrollment only)
- Rectal Sponges
  - Pharmacodynamics (PD)
  - Mucosal Immunology (MI)*
  - Pharmacokinetics (PK) is not collected at this visit. Why?

Collection of Rectal Specimens

- HPV
  - Qiagen Digene Female Swab Collection Kit (Catalog No. 5123-1220)
  - Place swab in vial and snap shaft at score line. Wrap lid with parafilm. Storage at ≤-70°C.
  - Log into LDMS and batch ship to MTN LC along with LDMS shipping manifest.
  - Ship the tube on dry ice. Use diagnostics packing code 650, UN3373.

Collection of Rectal Specimens

- Rectal Sponges for PD, PK and MI*
  - Use gloves when handling sponges. Mark each sponge and microtube to identify set.
  - Tare a calibrated weighing scale and weigh each microtube + sponge. Document weight on LDMS TS.
  - After specimen collection, re-weigh the sponge + microtube on the same scale previously used. Document weight.
  - Transport on ice and freeze at ≤-70°C within 2 hours of collection. Record freeze time.
  - Batch ship on dry ice when notified by MTN LC.

Collection of Rectal Specimens*

- Mucosal Gene Expression Array
  - 2 biopsies – 1 biopsy in RNA*later* per cryotube (blue top)
  - Refrigerate (2-4°C) overnight (16-24hr) then freeze at ≤-70°C.
  - Batch ship on dry ice to MTN LC
- Histology
  - 1 biopsy in 10% formalin (orange top tube)
  - Submit to local lab for embedding

Collection of Rectal Specimens*

- PD
  - 2-4 biopsies collected in biopsy transport media.
  - Deliver to processing lab within 30 minutes of collection.
  - Follow MTN LC SOP for ex vivo challenge.
- Mucosal T Cell Phenotyping
  - 7 biopsies in 12-15ml transport media.
  - Deliver to processing lab for testing.
- Proteomics
  - 1 biopsy snap freeze (dry ice bath or LN_2) at ≤-70°C (green top)

Order of Collection for Rectal Samples

Rectal samples should be collected in this order:

- Anal swab for HSV 1/2
- Anal swab for HPV
- Rectal swab for GC/CT
- Rectal sponges for PD and PK

Rectal samples for Tissue/Fluid subset only

- Rectal sponge for mucosal immunology
- Biopsies* for PK, PD, Proteomics, Histology, Mucosal T cell phenotyping, and Mucosal Gene Expression Array

Priority for Rectal Biopsies

If at anytime collection of biopsies are limited submit testing in order of priority. Section 10.7 MTN 017 SSP - Testing of Rectal Specimens

- PK (2-5)
- Mucosal Gene Expression Array (2)
- Histology (1)
- PD (2-4)
- T Cell Phenotyping (7)
- Proteomics (1)

If still unsure, contact the MTN 017 LC representative and management team

SCHARP

HCR-1, Page 1 of 1

SAMPLE: DO NOT FAX TO DATAFAX

MTN-017 (198)

HCR-1 (330)

Visit Code ☐☐.☐ 1

Participant ID ☐☐☐-☐☐☐☐☐-☐

Site Number | Participant Number | Chk

HIV Confirmatory Results

Specimen Collection Date ☐☐ dd ☐☐☐ MMM ☐☐ yy

1. HIV Western Blot — Not done/Not collected ☐ (Go to item 3.) | negative ☐ | positive ☐ | indeterminate ☐ (If negative or indeterminate, notify Network Lab.)

2. HIV Western Blot band results

Western Blot Band	(-) Negative	(+/-) Indeterminate	(+) Positive	(++) High Positive
	Band Interpretation			
GP160	☐	☐	☐	☐
GP120	☐	☐	☐	☐
P65	☐	☐	☐	☐
P55/51	☐	☐	☐	☐
GP41	☐	☐	☐	☐
P40	☐	☐	☐	☐
P31	☐	☐	☐	☐
P24	☐	☐	☐	☐
P18	☐	☐	☐	☐

2a. Were any other bands present? ☐ yes ☐ no

3. HIV RNA PCR — Not done/Not collected ☐ (Go to item 4.)

Alternate Collection Date: ☐☐ dd ☐☐☐ MMM ☐☐ yy

☐ > ☐ = ☐ < ☐☐☐☐☐☐☐ viral copies/mL OR ☐ target not detected

3a. RNA PCR kit lower limit of detection: ☐ 20 ☐ 40 OR ☐☐☐ viral copies/mL

4. Absolute CD4+ — Not done/Not collected ☐ (Go to item 5.)

Alternate Collection Date: ☐☐ dd ☐☐☐ MMM ☐☐ yy

☐ unable to analyze OR ☐☐☐☐ cells/mm^3

4a. CD4% ☐ not available OR ☐☐.☐ %

5. Final HIV Status: ☐ HIV-uninfected ☐ HIV-infected ☐ pending

Comments:

☐ ☐ ☒ ☐ 26-APR-13

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Staff Initials/Date

HIV Confirmatory Results (HCR-1)

Purpose: This form is used to document results from local lab confirmatory HIV testing if and when a participant has a positive rapid or EIA HIV test result during follow-up. This form also documents the HIV RNA viral load and CD4+ count obtained on the day the participant has a positive rapid or EIA HIV test result.

General Information/ Instructions: Complete this form for each visit where the participant has at least one positive rapid or EIA HIV test. As a reminder, the MTN-017 Follow-up HIV Testing Algorithm is as follows:

- **STEP 1:** Perform two rapid or one EIA HIV test(s). If at least one result is positive, go to STEP 2.
- **STEP 2:** Collect blood on same day as positive rapid or EIA HIV result and perform the following:
  - Western Blot testing and record results in items 1 and 2.
  - Viral load and CD4+ testing. Record results in items 3 and 4.
  - Store plasma. Document date stored plasma was collected on the HIV Results CRF (item 4a).

Fax this form to SCHARP DataFax as soon as any results are available, leaving all pending items blank. Do not wait for all results before faxing. Faxing this form with items blank will not generate a QC.

Item-specific Instructions:

- **Visit Code:** The visit code recorded on this form should be the same visit code recorded on the HIV Results form documenting the positive HIV rapid or EIA test result.
- **Specimen Collection Date:** Record the date the specimen was collected (NOT the date results were reported or recorded on the form). The Specimen Collection Date should be the same date as the collection date of the plasma for HIV confirmatory testing (HIV Results form, item 4a).
- **Alternate Collection Date:** This date is to be completed ONLY if the specimen was collected on a date after the Specimen Collection Date. A specimen collected for the same visit but on a different date should be recorded on the same form.
- **Not done/Not collected:** Mark this box in the event that a specimen was not collected or if the specimen was collected, but a result is not available due to specimen loss or damage. Record the reason why the result is not available in Comments.

Items 3 and 4: If, based on these results, the Network Lab requests Western Blot testing to be repeated, complete a new HIV Results form (items 4 and 4a) to document the WB specimen collection. Also complete a new HIV Confirmatory Results form for the repeat WB results.

Item 3: Record the participant's HIV RNA PCR result exactly as it appears on the lab report source document, regardless of whether the result is more or less than the limit of detection for the assay.

Item 4a: If automatically calculated, record the CD4+ percentage that was reported for the specimen in item 4. If the CD4+ percentage is not available (was not reported and would have to be manually calculated), mark the "not available" box.

Item 5: Once a participant's HIV status has been determined, record the final HIV status. If the final HIV status is not clearly negative or clearly positive once all results are available, mark the "pending" box. If the participant's final HIV status is determined to be positive (according to the protocol testing algorithm), update the Clinical Product Hold/Discontinuation Log to reflect permanent discontinuation of study product.

SAMPLE: DO NOT FAX TO DATAFAX

MTN-017 (198)

SS-1 (149)

Visit Code ☐☐.☐ 1

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

Specimen Storage

Initial Specimen Collection Date ☐☐ dd ☐☐☐ MMM ☐☐ yy

	Alternate Collection Date dd	MMM	yy	not required	stored	not stored	Reason:
1. PBMC for PK:	☐☐	☐☐☐	☐☐	☐	☐	☐→	_____
2. Plasma for PK:	☐☐	☐☐☐	☐☐	☐	☐	☐→	_____
3. Anal swab for anal HPV typing:	☐☐	☐☐☐	☐☐	☐	☐	☐→	_____
4. Rectal sponge for adherence PK:	☐☐	☐☐☐	☐☐	☐	☐	☐→	_____
5. Rectal sponge for PD:	☐☐	☐☐☐	☐☐	☐	☐	☐→	_____

	dd	MMM	yy	24-hour clock hr : min	not required	
6. Date and time of last dose:	☐☐	☐☐☐	☐☐	☐☐ : ☐☐	OR ☐→	*If not required, end of form.*

6a. Is the date/time of last dose a best estimate, or did the participant provide source documentation? best estimate ☐ source documentation ☐

Specimen Storage (SS-1)

Purpose: This form is used to document collection and storage of plasma, PBMC, and rectal specimens by the local site laboratory during Enrollment and follow-up.

General Information/ Instructions: Complete this form at Enrollment, at each regularly scheduled follow-up visit, and, if the participant terminates the study early, the Early Termination Visit.

- **Initial Specimen Collection Date:** Record the date that the first specimen was collected (NOT the date the results were reported or recorded on the form) for this visit. A complete date is required.
- **Alternate Collection Date**: This date is to be completed ONLY if the specimen was collected on a date after the Initial Specimen Collection Date. A specimen collected for the same visit but on a different date should be recorded on the same form. A complete date is required.

Item-specific Instructions:

Visit Code: Record the visit code assigned to this visit. Refer to the Study-Specific Procedures (SSP) Manual for more specific information on assigning visit codes.

Items 1–5: If the specimen was not collected because it was not required at this visit, mark the "not required" box. If the specimen was required to be stored, but for some reason it was not stored, mark the "not stored" box and record the reason on the line provided.

Item 6: Documentation of the date and time of last dose is required at each Mid-period and End Period Visit. Only mark the "not required" box at Enrollment, Initiate Period, and Interim Visits.

Item 6a: Only mark "source documentation" if the participant provided written documentation of the actual date and time s/he used his/her last dose of study product prior to the visit. Otherwise, mark "best estimate."

SCHARP

RBF-1, Page 1 of 1

MTN-017 (198) RBF-1 (130)

Visit Code . 1

Participant ID

Site Number - Participant Number - Chk

Rectal Biopsy/ Fluid Subset Specimens

Initial Specimen Collection Date

dd MMM yy

Alternate Collection Date

1. Rectal sponge for mucosal immunology: dd MMM yy — not required ☐ stored ☐ not stored ☐ → Reason: _____

2. Was a sigmoidoscopy performed at this visit? yes ☐ no, specify: _____ ☐ → *End of form.*

3. Sigmoidoscopy findings: no abnormal findings ☐ abnormal findings ☐

3a. Abnormal sigmoidoscopy findings: *Mark all that apply.* → *If no abnormal findings, go to item 4.*

☐ Erythema	☐ Friability	☐ Polyps
☐ Abnormal vessels	☐ Bleeding	☐ Hemorrhoids
☐ Ulceration	☐ Discharge	☐ Other abnormal findings, specify: _____

At Enrollment, evaluate any abnormalities for eligibility. Update Pre-existing Conditions when applicable.
During follow-up, complete or update AE Log when applicable.

Alternate Collection Date

	dd	MMM	yy	not required	stored	not stored	Reason:
4. Rectal biopsies for PK:				☐	☐	☐ →	_____
5. Rectal biopsies for PD:				☐	☐	☐ →	_____
6. Rectal biopsies for mucosal T-cell phenotyping:				☐	☐	☐ →	_____
7. Rectal biopsies for mucosal gene expression:				☐	☐	☐ →	_____
8. Rectal biopsy for histology:				☐	☐	☐ →	_____
9. Rectal biopsy for proteomics:				☐	☐	☐ →	_____

☐ ☐ ☐ ☒ 12-NOV-12

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Staff Initials/Date

Rectal Biopsy/ Fluid Subset Specimens (RBF-1)

Purpose: This form is used to document collection and storage of rectal biopsy and fluid by the local site laboratory for those participants in the PK/PD/mucosal immunology subset only. It is also used to document the findings identified via flexible sigmoidoscopy.

General Information/ Instructions: Complete this form for participants in the PK/PD/mucosal immunology subset only at Enrollment and at each end-of-period visit. If the participant terminates the study early, complete this form at the Early Termination Visit.

- **Initial Specimen Collection Date:** Record the date that the first specimen was collected (NOT the date the results were reported or recorded on the form) for this visit. A complete date is required.
- **Alternate Collection Date**: This date is to be completed ONLY if the specimen was collected on a date after the Initial Specimen Collection Date. A specimen collected for the same visit but on a different date should be recorded on the same form. A complete date is required.

Item-specific Instructions:

Visit Code: Record the visit code assigned to this visit. Refer to the Study-Specific Procedures (SSP) Manual for more specific information on assigning visit codes.

Items 1 and 4–9: If the specimen was not required to be collected at this visit, mark "not required." If the specimen was required to be stored, but for some reason it was not stored, mark "not stored" and record the reason on the line provided.

Item 3a: Mark the box to the left of each abnormal finding observed. If an observed abnormal finding is not listed, mark "Other abnormal findings, specify" and describe the abnormal finding on the line provided.

Any Questions?