



# MTN 026 Laboratory Training Enrollment Visit

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# Objectives



- ◆ Overview of Lab testing
- ◆ Collection of Pelvic Samples
- ◆ Collection of Anorectal Specimens
- ◆ Specimen Management
- ◆ Q&A

# Overview of Lab Testing by Visit

	VST 1 SCR	VST 2 ENR	VST 3 Dose	VST 4-6	VST 7-12	VST 13 Final Dose	VST 14-16
UA and Culture	★	★	★	★	★	★	★
Urine GC/CT	X	★	★	★	★	★	★
Urine hCG (♀)	X	X			7		14
CBC/diff/plt	X	★	★	★	★	★	★
AST/ALT/Creatinine	X	★	★	★	★	★	16
HIV-1 and HIV-2 serology	X	X			7		16
Syphilis Serology	X	★	★	★	★	★	★
HSV-1, HSV-2, HBsAg, and HCV Serologies	X						
Coagulation (PT/INR)	X						
Plasma Archive/Storage		X			7		16
Plasma PK (Vsts 3 and 13: pre and 30-60 or 120 mins)			◆	X	7 and 8	◆	X

★ As indicated, ◆ Randomized assigned time points

# Overview of Lab Testing by Visit

	VST 1 SCR	VST 2 ENR	VST 3 Dose	VST 4-6	VST 7-12	VST 13 Final Dose	VST 14-16
Vaginal NAAT for GC/CT (♀)	X						
CVL for PD/PK (♀)		X		♦		X	♦
CVF and Biopsies for PK (♀)				♦		X	♦
Pap Test (♀)	★						
Rectal HSV-1/2 detection	★	★	★	★	★	★	★
Rectal NAAT for GC/CT	X	★	★	★	★	★	★
Rectal Fluid for Microflora		X	♦			♦	
Rectal Sponge Mucosal Safety		X	♦			♦	
Rectal Fluid and Biopsies for PK (Vsts 3 and 13: 30-60 or 120 mins)			♦	♦	7 and 8	♦	♦
Rectal Enema for PD/PK		X		♦			♦
Rectal Biopsies for PD (Vsts 3 and 13: 30-60 or 120 mins)		X	♦	♦		♦	♦
Rectal Biopsies for GE, Histology, T Cell Pheno, and Proteomics		X	♦			♦	

★ As indicated, ♦ Randomized assigned time points

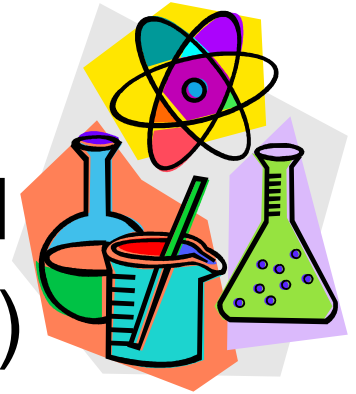


# Urine Specimens

- ◆ Urine hCG (♀)
- ◆ Urinalysis and culture (if indicated)
- ◆ NAAT for GC/CT (if indicated)

# Blood Specimens

- ◆ Chemistries, Syphilis Serology, and CBC w/ diff and plts (if indicated)



- ◆ HIV Testing (prior to enrollment)

- ◆ Follow testing algorithm

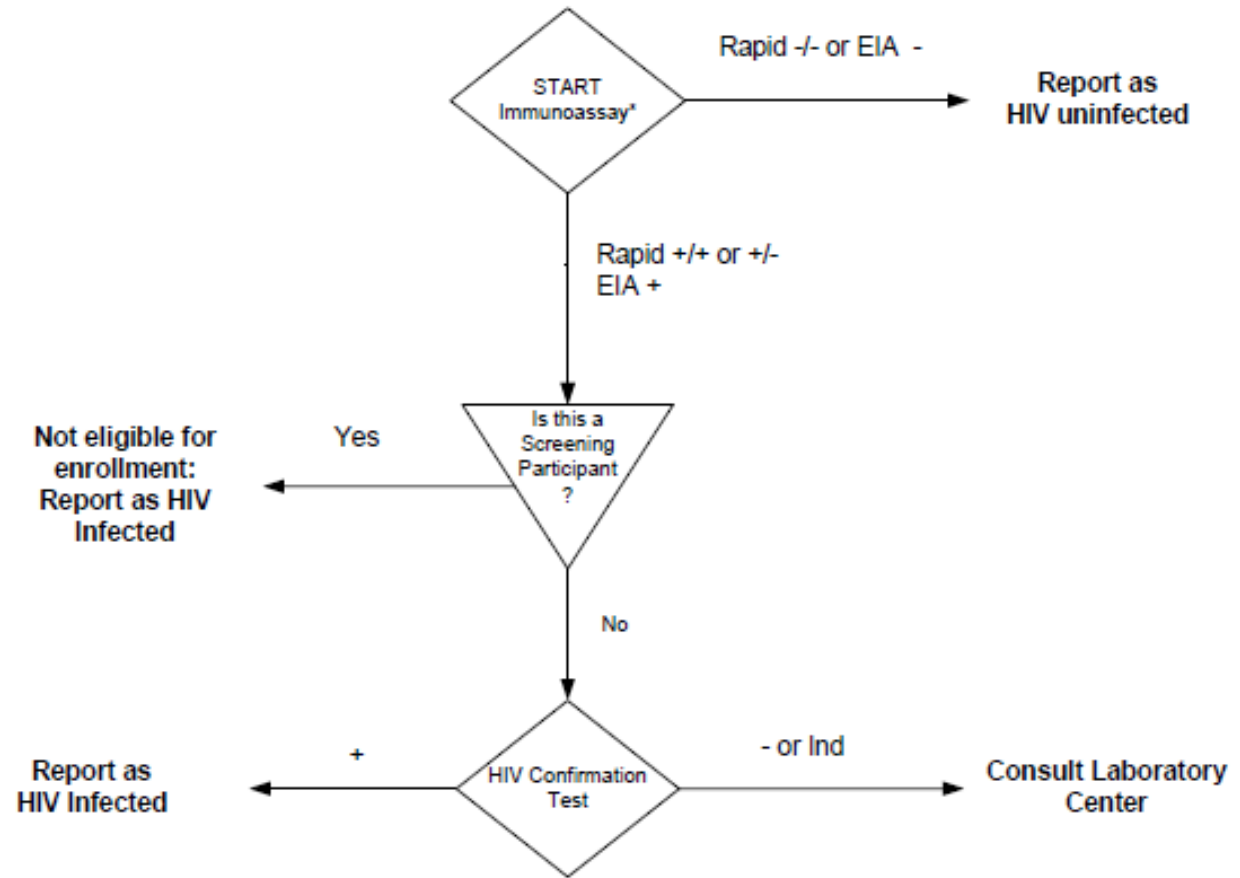
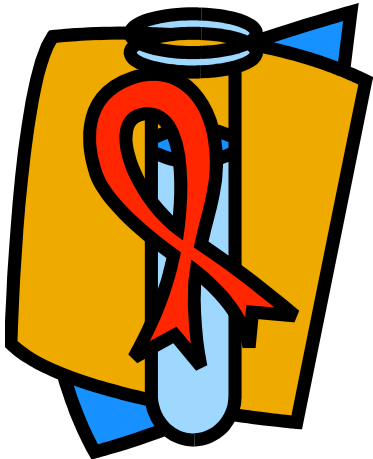
- ◆ Plasma Archive (baseline)/Storage

- ◆ Plasma archive is collected at enrollment.

- ◆ Freeze plasma within 4 hours if held at RT. If refrigerated or on ice, freeze within 24 hours.



# HIV Testing Algorithm



\*CLIA certified labs may perform 1 rapid test  
Ind: Indeterminate test results  
EIA: Enzyme Immunoassay

# Pelvic Samples (♀)

- ◇ Cervicovaginal Lavage for baseline PD
  - ◇ 10mL of normal saline should be used to lavage the cervix, fornices, and vaginal walls. Using a syringe collect all of the CVL and place into a 15mL conical tube.
  - ◇ CVL specimens are kept on wet ice or refrigerated and should be processed within 8 hours of collection.





# Rectal Specimens

- ❖ Rectal Swabs
  - ❖ Microflora
  - ❖ NAAT for GC/CT (if indicated)
  - ❖ HSV-1 and -2 (if indicated)
- ❖ Rectal Sponge for Mucosal Safety
- ❖ Rectal Enema for PD/PK
- ❖ Rectal Biopsies in order of importance
  - ❖ Gene Expression
  - ❖ Histology
  - ❖ PD
  - ❖ T Cell Phenotyping
  - ❖ Proteomics



# Collection of Rectal Specimens

## ❖ Rectal Swab for Microflora

- ❖ Collect the specimen for microflora by rotating a flocced nylon swab several times over the lateral wall of the rectum. Insert the swab into a cryovial and snap the shaft of the tube off in order to screw on the top.
- ❖ Keep refrigerated and freeze within 2 hours of collection.



# Collection of Rectal Specimens

- ❖ Rectal Sponges for Mucosal Safety
  - ❖ Weigh sponge before and after collection using the same analytical scale measuring to 0.1mg. Record weights onto LDMS Tracking Sheet.
  - ❖ Use transfer pipette (See Clinical Considerations Section 8 of SSP) to hold sponge against lateral wall for 2 minutes.
  - ❖ Transport on ice and freeze at  $\leq -70^{\circ}\text{C}$  within 2 hours of collection. Record freeze time.



# Collection of Rectal Specimens

## ❖ Rectal Enema for PD

- ❖ In a conical tube collect 10 mLs of the rectal enema.
- ❖ Rectal enema should be kept on wet ice or refrigerated and processed within 8 hours of collection.

## ❖ Rectal Biopsies for PD

- ❖ Four biopsies for PD should be collected and placed into biopsy transport media immediately.
- ❖ Transport biopsies to lab within **15-30 minutes** from time of collection.



# Collection of Rectal Specimens

## ❖ Rectal Biopsies for Gene Expression

- ❖ Collect two (2) biopsies.
- ❖ Place each biopsy into a labeled cryovial containing 1.5 mL of *RNAlater* (Ambion, Invitrogen Cat #AM7020). Be sure the biopsy is submerged.
- ❖ Store at 4°C overnight (16-24 hours) then transfer to  $\leq -70^{\circ}\text{C}$  for storage.

## ❖ Rectal Biopsy for Histology

- ❖ Collect one (1) biopsy and place into a cryovial containing 10% formalin.
- ❖ Store at room temperature.



# Collection of Rectal Specimens

- ❖ Rectal Biopsies for T Cell Phenotyping
  - ❖ Collect seven (7) biopsies and submerge into 10-15mL of transport media. Keep refrigerated.
  - ❖ **For UAB:** Ship specimen First Overnight on ice to the McGowan lab for processing.
  - ❖ **For Pitt and Bangkok:** Place specimens in the refrigerator and allow to rest overnight prior to processing according to the McGowan Lab SOP.
  
- ❖ Proteomics
  - ❖ Collect one (1) biopsy and place into a cryovial.
  - ❖ Snap freeze at  $\leq -70^{\circ}\text{C}$  within 2 hours of collection.



# Specimen Management

- ◆ All specimens must be tracked according to site Chain of Custody.
- ◆ CRFs are required for specimens reported to SCHARP.
- ◆ Specimens to be shipped to the Laboratory Center must be accompanied by an LDMS tracking sheet and entered into LDMS.

# LDMS Tracking Sheets

# of TUBES or SPECIMENS	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQUOT DERIVATIVE	ALIQUOT SUB ADD/ DER	INSTRUCTIONS FOR PROCESSING
<input type="checkbox"/>	Rectal Swab – <i>Microflora</i> (REC) Collection Time: _____:_____ Hour : Min	NON	SWB	N/A	Time Frozen: _____:_____ Hour : Min Freeze at $\leq -70^{\circ}\text{C}$ within 2 hours of collection. Enter MF into Other Spec ID field of LDMS.
<input type="checkbox"/>	Rectal Swab – PK (REC) Collection Time: _____:_____ Hour : Min	NON	SWB	N/A	$\frac{\text{Post-weight}}{\text{Pre-weight}} = \frac{\text{Net weight}}{\text{mg}}$ Time Frozen: _____:_____ Hour : Min Freeze at $\leq -70^{\circ}\text{C}$ within 2 hours of collection. Enter PK into Other Spec ID field of LDMS.
<input type="checkbox"/>	Rectal Sponge – <i>Mucosal Immunology</i> (REC) Collection Time: _____:_____ Hour : Min	NON	SPG	N/A	$\frac{\text{Post-weight}}{\text{Pre-weight}} = \frac{\text{Net weight}}{\text{mg}}$ Time Frozen: _____:_____ Hour : Min Freeze at $\leq -70^{\circ}\text{C}$ within 2 hours of collection.
<input type="checkbox"/>	Rectal Enema – PD (REC)	NSL	FLD	N/A	Store supernatants in aliquots of 1.0 ml. Freeze at $\leq -70^{\circ}\text{C}$ within 8 hours of collection.
			PEN	NSL	Suspend in 0.5ml of normal saline and freeze at $\leq -70^{\circ}\text{C}$ within 8 hours of collection.
<input type="checkbox"/>	Rectal Biopsies – PD (FSR) Collection Time: _____:_____ Hour : Min	BTM	BPS	N/A	1 _____:_____ = _____ mg 2 _____:_____ = _____ mg 3 _____:_____ = _____ mg

24-hour clock

Note: Weights are recorded in equation format.



Any Questions?

