



MTN-038

Laboratory Training

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MTN Laboratory Center

Magee-Womens Research Institute

Pittsburgh, PA

Objectives

- Resources
- Specimen Management
- Overview of Lab testing
- Supplies
- Q&A

Resources

- MTN LC: Magee-Womens Research Institute
 - May Beamer: 412-641-6042 mbeamer@mwri.magee.edu
 - Michele Austin 412-641-6047 maustin@mwri.magee.edu
 - Pam Kunjara, Wayne Hall, Ted Livant
- SSP Section 10: Lab Considerations
 - Most current version
 - <https://mtnstopshiv.org/research/studies/mtn-038>
 - Lab SSP Tables

Specimen Management slide 1

➤ Specimen Labeling (Section 10.2)

- SCHARP-provided label for primary specimen
 - Include Collection date and Visit code.
 - If information is handwritten, use indelible ink.
- LDMS labels for aliquots stored for MTN LC.
 - Please do not remove SCHARP label.

➤ Special Circumstances (Section 10.3)

- Collection of a specimen will be repeated if samples cannot be tested (Ex. Purple Top broke on transit to the lab).
- A protocol deviation (PD) form may be required in cases when additional specimens are collected either due to a laboratory or clinic error.

Specimen Management slide 2

- Chain of Custody SOP
 - Site activation checklist
 - Current CLIA Certification for testing laboratories
 - » Safety
 - Hematology, Chemistries
 - » STI, Pregnancy
 - Specimen Handling
 - Initials or signatures, date and time received/delivered
 - Local Lab Requisitions
 - Medidata Rave, eCRFs
 - » Collection of samples
 - Laboratory Data and Management System (LDMS) Tracking Sheet
 - » Receipt of samples for storage of MTN
 - » If edit, MTN LC /FHI review to ensure that all samples are captured

LDMS (Section 10.4)

- LDMS Tracking Sheet
 - Samples stored for MTN LC
 - Tables 10-3 and 10-4
 - On tracking sheet, but not logged into LDMS
 - Freeze times to ensure within storage parameters
 - If clinic places samples on dry ice, then clinic completes the time
 - Pre- and post- weights for vaginal PK and rectal PK swabs, cx biopsy for PK (not PD)
 - Make sure post weight is larger
- LDMS Log In
 - Net Weight for CVF PK, Rectal PK or CX Biopsy PK
 - The average weight for vag PK is ~81 mG (65-115 mG)
 - LDMS Monitoring Reports
 - Monthly check for discrepancies (vs RAVE eCRFs)
 - Resolution within 2 weeks
 - Collection times
 - Time points for PK samples (Enrollment, Day 91/PUEV)

Urine Specimens

- Specimen collection (10.5.1)
- hCG (Section 10.5.2)
 - Quidel Quickvue or SureVue; Beckman Coulter ICON 25
 - Required at Screening, Enrollment, Days 28, 56, and 91/PUEV
 - *Pregnancy Test Result CRF*
- Dipstick urinalysis (Sections 10.5.3)
 - Roche Chemstrip 5OB test strips; Siemens Multistix 10 SG or Uristix 4
 - If clinically indicated, leukocytes and nitrites
 - Urine culture, proceed per local standard of care
 - *Local Laboratory Results CRF*

Blood Specimens slide 1

(SSP Table 10-1)

	Visit 1 SCR	Visit 2 ENR (Day 0)	Visit 3 (Day 1)	Visit 4 (Day 7)	Visit 5 (Day 14)	Visit 6 (Day 28)	Visit 7 (Day 42)	Visit 8 (Day 56)	Visit 9 PUEV/Early Termination (Day 91)	Visit 10 Final Contact (Day 92)
BLOOD	HIV-1/2 testing	X	X	*	*	*	*	*	X	
	Plasma for archive		X							
	AST/ALT	X					*	*	X	
	CBC with differential and platelets	X	*	*	*	*	*	*	X	*
	Serum creatinine	X	*	*	*	*	*	*	X	*
	Hep B surface antigen	X								
	Syphilis serology	X		*	*	*	*	*	*	
	HSV 1/2 serology		X							
	TFV levels			X	X	X	X		X	0-,4-HR Post

Blood Specimens slide 2

- Complete Blood Count with Differential and Platelets (Section 10.6.2)
 - Required at Screening and Day 91/PUEV
 - If clinically indicated, other visits
 - *Hematology CRF*
- Chemistries: AST & ALT, Creatinine (Section 10.6.2)
 - Required at Screening and Day 91/PUEV
 - If indicated, other visits
 - *Local Laboratory Results CRF*

Blood Specimens slide 2

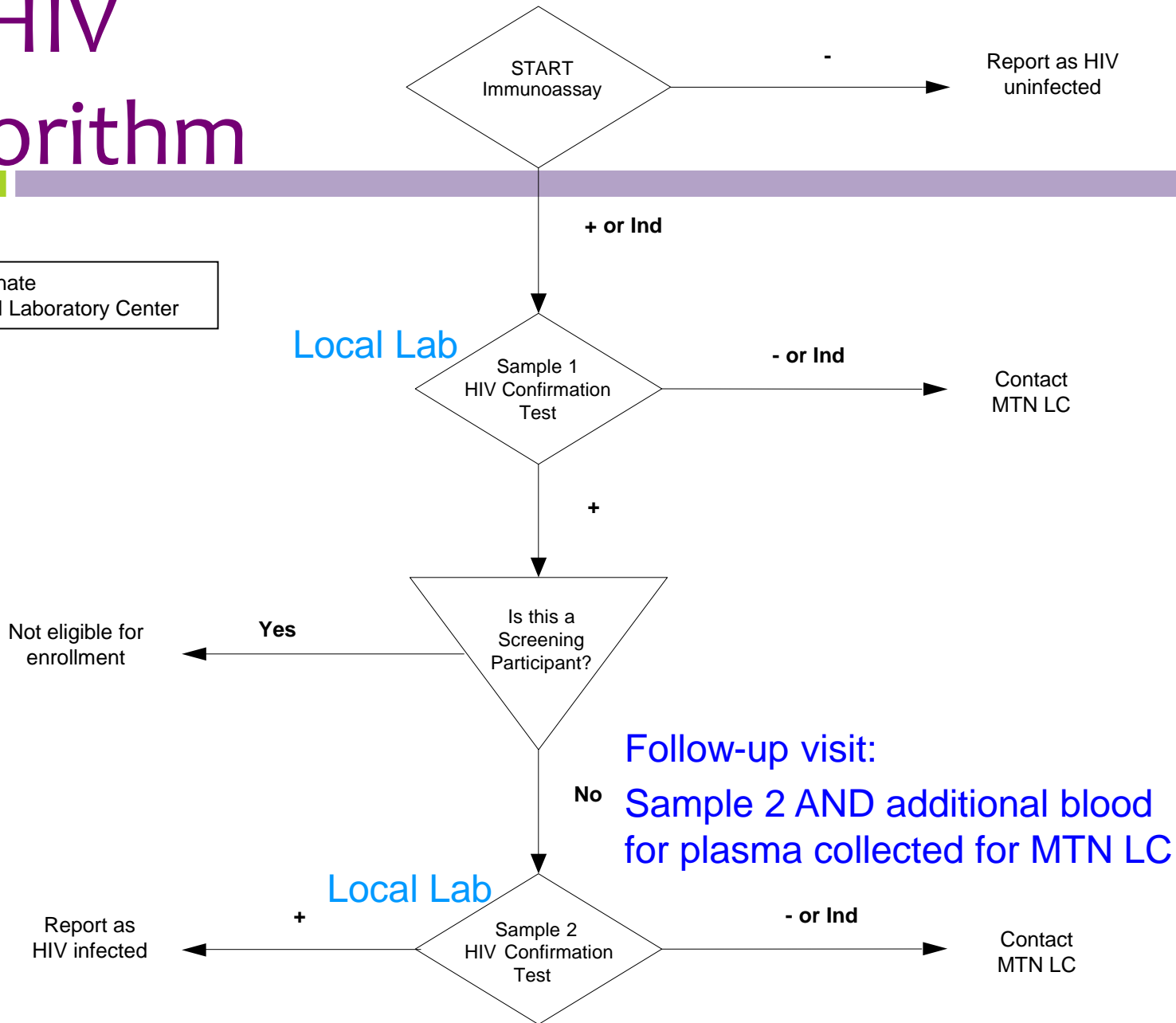
- Syphilis Serology (Section 10.6.5)
 - Required at Screening
 - If clinically indicated at follow-up visits
 - *STI Test Results CRF*
- *Hepatitis B Surface Antigen (10.6.3)*
 - Required at Screening
 - *STI Test Results CRF*
- *HSV 1/2 Serology (10.6.7)*
 - Used with CVF for anti-HSV-2 activity testing
 - Only at Enrollment
 - Collect serum, at least two aliquots, ≥1-mL in first tube, remainder in the other.
 - Store both aliquots, side by side – in same box
 - Batch shipment at end of study for testing by MTN LC
 - *LDMS Tracking Sheet, Specimen Storage CRF*

Blood Specimens

- Plasma archive (Section 10.6.6)
 - *LDMS Tracking Sheet, Specimen Storage CRF*
 - Required at Enrollment
 - Storage: 1.5-mL aliquots; $\leq -70^{\circ}\text{C}$ within 24 hrs (4°C) or 4 hrs (RT°)
- HIV-1/2 Testing (Section 10.6.4)
 - Required at Screening, Enrollment, Day 91/PUEV
 - If clinically indicated, follow-up visits
 - *HIV Results CRF*
 - *HIV Confirmation CRF*
 - If indicated at follow-up, additional sample collected with SAMPLE 2 in HIV Algorithm
 - Viral load and HIV drug resistance testing by MTN Virology Core

HIV Algorithm

Ind: Indeterminate
MTN LC: MTN Laboratory Center



Blood Specimens, Stored for MTN LC

- Plasma for TFV PK (Section 10.6.8)
 - LDMS Tracking Sheet, Specimen Storage CRF
 - Days 1, 7, 14, 28, 56, Final
 - Day 91/PUEV: 0-HR before ring removed
 - Timer towards 4-HR collection point starts after ring removal
 - Storage: ≥ 1 -mL aliquots; $\leq -70^{\circ}\text{C}$ within 8 hours
 - Primary Plasma PK set; Backup Plasma PK set

# of TUBES or SPECIMENS	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQUOT DERIVATIVE	ALIQUOT SUB	INSTRUCTIONS FOR PROCESSING LAB
	0-HR Blood (BLD) PK Collection Time ____ : ____ hour : min	EDT (purple top)	PL1 or PL2	N/A	Single spin: 1300xg, 10 minutes OR Double spin: 800xg, 10 minutes. Place plasma in a tube to spin again at 800xg for 10 minutes. Label two cryovials and aliquot ≥ 1.5 -mL of plasma into each cryovial. Store plasma at $\leq -70^{\circ}\text{C}$ within 8 hours of collection.

Blood Specimens, Stored for MTN LC

Table 10-4 LDMS Specimen Management Guide to Logging in MTN-038

Sample	Primary Specimen	Primary Derivative	Aliquot Derivative	Aliquot Sub additive/ derivative	Other Specimen ID	# of Aliquots	Aliquot Volume	Units	Time or Weights
Plasma for Archive or Confirmatory Test	BLD	EDT	PL1 (single spin)	N/A	CON (follow-up)	2-5	≥1.5 mL in 2-mL cryovials	mL	--
Plasma for PK (TFV)			PL2 (double spin)		PK	2-5	≥1.0 mL in 2-mL cryovial	mL	See 10.4.2
Serum for HSV-1/2 Testing	BLD	NON	SER	N/A		1-2	≥1.0 mL in 2-mL cryovial	mL	--

Rectal Fluid Swab for PK (Section 10.9)

- Days 1, 14, 28, 56, and 91 (0-, 4-HR)
- Collection with anoscope
 - Within 30 minutes of blood collection
 - Hold swab against mucosa, 2 minutes
- Similar weighing procedure as vaginal swab
- *LDMS Tracking Sheet, Specimen Storage CRF*
- Storage within 2 hrs, $\leq -70^{\circ}\text{C}$

# of TUBES or SPECIMENS	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQOT DERIVATIVE	ALIQOT SUB	INSTRUCTIONS FOR PROCESSING LAB			
	4-HR Rectal Fluid (REC) for PK Collection Time ____ : ____ hour : min	NON	SWB	N/A	Pre (before sample collection) and Post (after sample collection) weights are needed. May be kept on ice until frozen. Freeze at $\leq -70^{\circ}\text{C}$ within 2 hours of collection.			
					Post-weight	Pre-weight	Net Weight	Freezing Time
					____ : ____ mg	____ : ____ mg	____ : ____ mg	____ : ____ hour : min

CVF Swab for PK_{slide 1} (10.7.6)

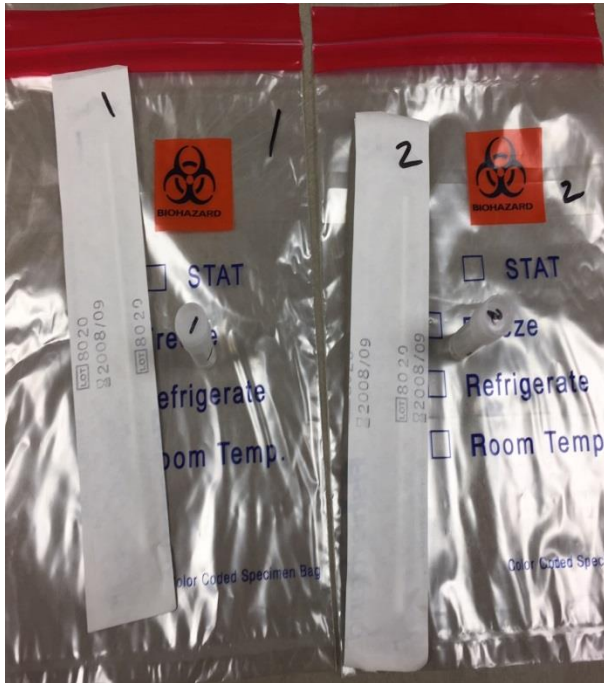
- Blood, Rectal swab (if applicable) for PK collected first
- Collected prior to speculum insertion
- Rectal and Vaginal swabs for PK collected within 30 minutes of PK blood collection
- Similar weighing procedure as rectal fluid swab
- *LDMS Tracking Sheet and Cervical Specimen Storage CRF*

# of TUBES or SPECIMENS	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQUOT DERIVATIVE	ALIQUOT SUB	INSTRUCTIONS FOR PROCESSING LAB			
	1-HR Vaginal Swab (VAG) for PK Collection Time ____:____ hour : min	NON	SWB	N/A	Pre (before sample collection) and Post (after sample collection) weights are needed. May be kept on ice until frozen. Freeze at $\leq -70^{\circ}\text{C}$ within 2 hours of collection.			
Post-weight					Pre-weight	Net Weight	Freezing Time	
____.____ mg					____.____ mg	____.____ mg	____:____ hour : min	

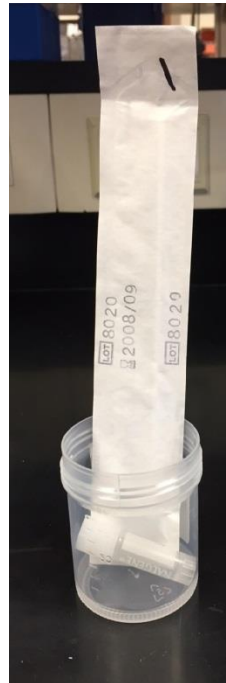
CVF Swab for PK slide 2 (10.7.6)

- Enrollment
 - Ring insertion, timer starts for 1- and 4-HR collection time target points
 - If collection delayed, no bearing on next time point
- Single time point
 - Days 1, 7, 24, 56, 92-Final Contact
- PUEV/Day 91
 - 0-HR, prior to ring removal and 4-HR
- If VR removed prior to visit, collect only single time point with blood for PK

Weighing Rectal and Vaginal Swabs for PK



Also have backup kits,
already weighed



Don't throw
wrapper or swab
shaft in garbage

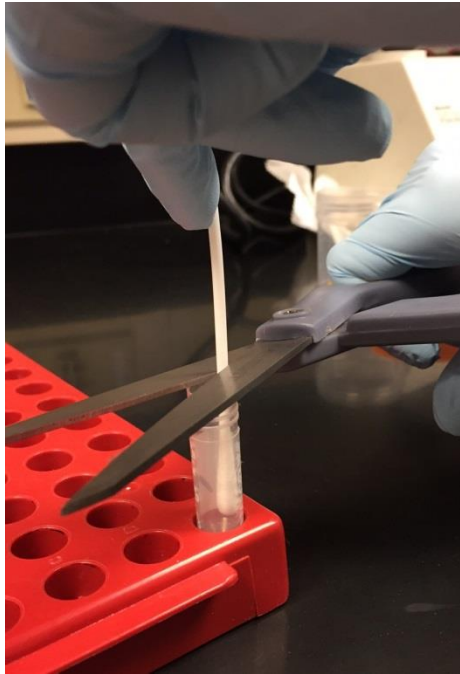


Weigh
everything

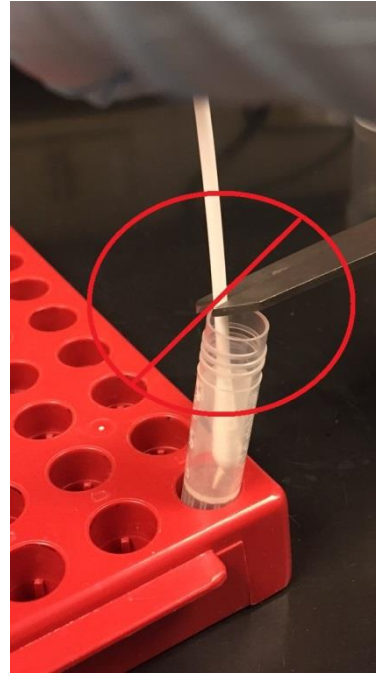
Use gloves when handling
items throughout collection



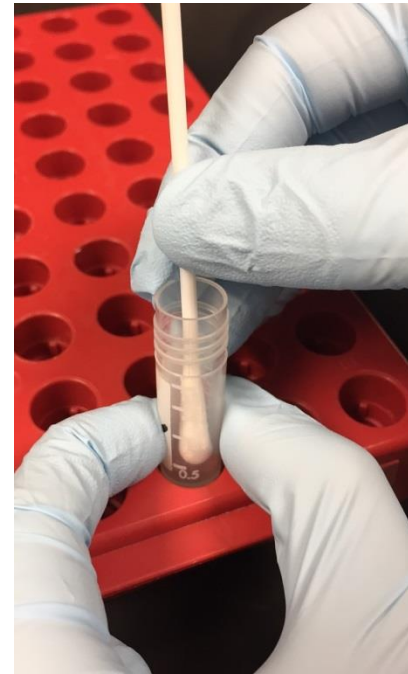
Cutting vs Bending Swab



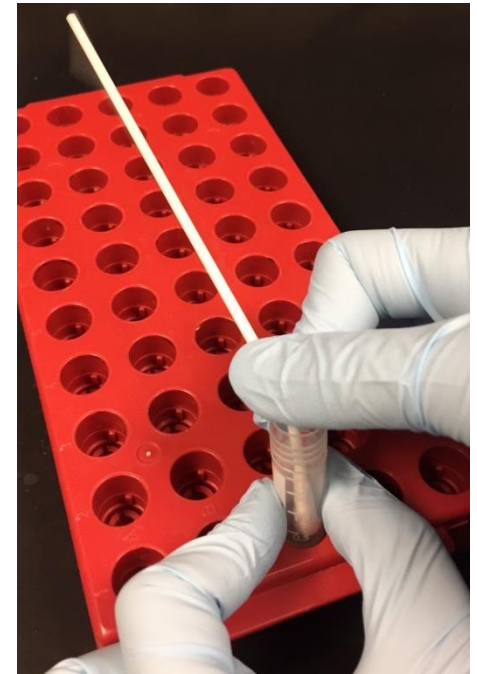
Easier cut



No leverage



Raise swab



Thumb over opening

Raise swab before cutting or bending for lid to close properly



Pelvic Exam Checklist slide 1

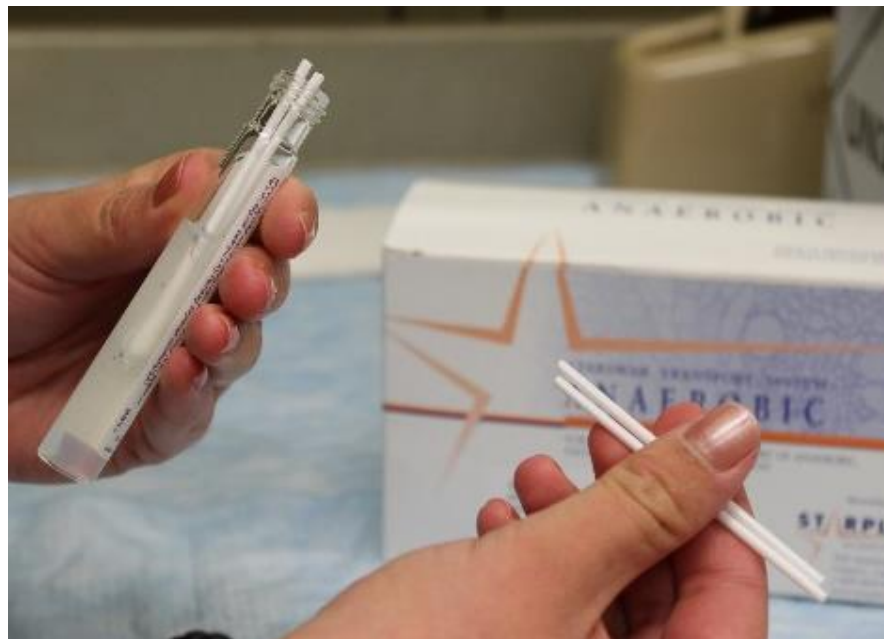
Vaginal Specimens

- *Trichomonas* and GC/CT NAAT – vaginal swabs (section 10.7.5)
 - Cepheid (2 tubes) vs GenProbe Aptima (1 tube) collection
 - Required at Screening; if clinically indicated at other visits
 - *STI Test Results CRF*
- qPCR for Microbiota (10.7.4)
 - 2 of the following: Flocked swab placed in a 2.0-mL cryovial
 - *Cervical Specimen Storage CRF, LDMS Tracking Sheet*
 - *Store in two separate boxes:*
 - *Primary qPCR box, Backup qPCR box*

Pelvic Exam Checklist slide 2

Quantitative Culture (10.7.3)

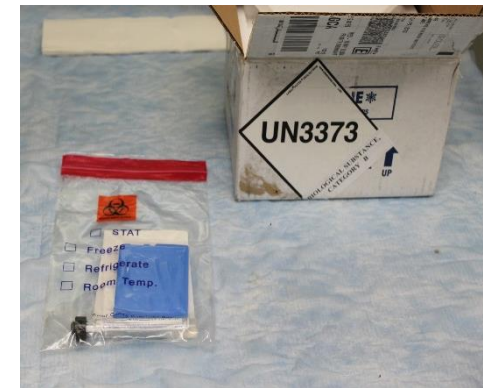
- Starplex Starswab Anaerobic Collection Kit, including 2 Dacron Swabs
 - Slowly insert swab into gel (approx. half way into gel, then break shaft)



Pelvic Exam Checklist slide 3

Quantitative Culture (10.7.3)

- Refrigerate (4°C) if not shipped immediately
- Ship multiple samples in one shipment with frozen ice packs
 - A separate biohazard specimen bag for each participant visit.
 - Wrap each tube in absorbent material (paper towel)
 - Place each tube, including corresponding vag slide in a case, in biohazard bag
- *Cervical Specimen Storage CRF, LDMS Tracking Sheet*
- Create batch shipment
- Email:
hillierlab@mwri.magee.edu



Pelvic Exam Checklist slide 4

Vaginal Specimens

- Bacterial vaginosis or candidiasis, if clinically indicated, any visit (section 10.7.2)
 - **Saline wet mount** for clue cells for BV
 - **KOH for whiff test** for BV
 - **KOH wet mount** for candidiasis
 - **pH for BV**
 - *STI Test Results CRF*
- Amsel's Criteria, must have at least 3 of the 4 following:
 - Thin homogeneous discharge
 - Amine odor
 - pH >4.5
 - >20% Clue cells

Pelvic Exam Checklist, slide 5

Vaginal Specimens

- Gram stain (Section 10.7.1)
 - Required at Enrollment, Days 28, 56, 91/PUEV
 - **One** swab, **ROLLED** onto two slides
 - Pencil to label frosted end
 - Affix SCHARP label under the frosted end
 - Affix LDMS label after it is made, over the pencil label
 - *Specimen Storage CRF and LDMS Tracking Sheet*
 - Storage
 - Primary VAG Gram Stain shipped with vaginal swab for quantitative culture
 - Duplicate VAG Gram Stain Set – stored on site



Pelvic Exam Checklist

CVF for Anti-HSV2 Activity

- Collection procedure (10.7.7)
 - Enrollment prior to ring insertion, Days 28 and 56
 - Starplex Scientific Starswabs Double Swab Dry kit
 - *LDMS tracking sheet, Cervical Specimen Storage CRF*

Sample	Primary Specimen	Primary Derivative	Aliquot Derivative	Aliquot Sub additive/ derivative	Other Specimen ID	# of Aliquots	Aliquot Volume	Units	Time or Weights
CVF/vaginal swab for PK	VAG	NON	SWB	N/A	PK	1	variable	mg	See 10.4.2 10.4.3
CVF for anti-HSV-2 activity	CVF	CTK	SWB	N/A	HSV-VG	2	1	Each	See 10.4.2
CVF for Biomarkers	CVF	NON	SWB	N/A		1	1	Each	See 10.4.2

- LDMS Laboratory (The SSP needs to be edited for this to be done by lab)
 - Each swab head will be placed and cut with sterile (autoclavable) scissors into a 2-mL cryovial labeled with LDMS label
 - The two cryovials stored side by side in same box.

Pelvic Exam Checklist

CVF for Biomarker

- Collection procedure (10.7.7)
 - All visits except Screening and Final Contact
 - Collect Dacron swab, place dry in 2-mL cryovial for $\leq -70^{\circ}\text{C}$ storage
 - *LDMS tracking sheet, Cervical Specimen Storage CRF*

Sample	Primary Specimen	Primary Derivative	Aliquot Derivative	Aliquot Sub additive/ derivative	Other Specimen ID	# of Aliquots	Aliquot Volume	Units	Time or Weights
CVF/vaginal swab for PK	VAG	NON	SWB	N/A	PK	1	variable	mg	See 10.4.2 10.4.3
CVF for anti- HSV-2 activity	CVF	CTK	SWB	N/A	HSV-VG	2	1	Each	See 10.4.2
CVF for Biomarkers	CVF	NON	SWB	N/A		1	1	Each	See 10.4.2

Pelvic Exam Checklist

Cervicovaginal Lavage (CVL)

- Collection procedure (10.7.9)
 - 10-mL normal saline
 - Collect CVL with syringe, place into 15-mL conical tube
- Collection schedule (Table 10-1)
 - Enrollment prior to ring insertion
 - PD and Biomarkers only
 - Days 28 and 56
 - PK , PD and Biomarkers

	Visit 1 SCR	Visit 2 ENR (Day 0)	Visit 3 (Day 1)	Visit 4 (Day 7)	Visit 5 (Day 14)	Visit 6 (Day 28)	Visit 7 (Day 42)	Visit 8 (Day 56)	Visit 9 PUEV/Early Termination (Day 91)	Visit 10 Final Contact (Day 92)
CVL for PK						X		X		
CVL for PD and biomarkers		X ^a				X		X		

- *LDMS tracking sheet, Cervical Specimen Storage CRF*

Pelvic Exam Checklist

Cervicovaginal Lavage (CVL)

- Sample preparation for storage
 - First centrifugation: ≥ 1 -mL aliquots of supernatant
 - Second centrifugation
 - Remove supernatant
 - Add 0.5-mL normal saline to the pellet
 - Storage @ $\leq -70^{\circ}\text{C}$ within 2 hours of collection

# of TUBES or SPECIMENS	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQUOT DERIVATIVE	ALIQUOT SUB	INSTRUCTIONS FOR PROCESSING LAB	
	Cervicovaginal Lavage (CVL) Collection Time ___:___ hour : min	NSL	FLD	N/A	CVL supernatant for biomarker: Place on ice or 4°C until transported to LDMS lab. Centrifuge at 800g for 10 minutes and without disturbing pellet, aliquot into 6-9 cryovials with a minimum of 1.0-mL in each. Freeze at $\leq -70^{\circ}\text{C}$ within 2 hours of collection.	Freezing Time
			CEN	NSL		CVL cell pellet: Re-spin at 800g for 10 minutes. Remove supernatant and resuspend pellet in 0.5-mL of normal saline and transfer into a single cryovial. Freeze at $\leq -70^{\circ}\text{C}$ within 2 hours of collection.

Pelvic Exam Checklist

Cervical Specimens (10.8)

- Pap Test per local standard of care
 - If indicated at Screening
- Cervical Biopsies
 - Participants are randomized to two groups for biopsy schedule
 - Days 14 and 56
 - Days 28 and 91/PUEV
 - For each group, PK samples (2 biopsies per visit) collected at both visits
 - For each group, PD samples (2 biopsies per visit) collected only at later visit
 - PD collected after PK biopsies (or if courier available) because of processing time
 - For Day 91/PUEV participants, collected prior to ring removal

	Visit 1 SCR	Visit 2 ENR (Day 0)	Visit 3 (Day 1)	Visit 4 (Day 7)	Visit 5 (Day 14)	Visit 6 (Day 28)	Visit 7 (Day 42)	Visit 8 (Day 56)	Visit 9 PUEV/Early Termination (Day 91)	Visit 10 Final Contact (Day 92)
Cervical biopsies for PK					X†	X†				
Cervical biopsies for PK and PD								X†	X†	

Pelvic Exam Checklist

Cervical Specimens (10.8)

- Cervical Biopsy FOR PK (10.8.2)
- LDMS tracking sheet, Cervical Specimen Storage CRF
- Two biopsies, one per cryovial
 - Label cryovials 1 and 2, markings on tubes prior to weighing
 - Collect biopsy, place biopsy in tube (no media), post-weight
 - Immediate placement of cryovial in dry ice ethanol bath or liquid nitrogen
 - Storage @ $\leq -70^{\circ}\text{C}$

# of TUBES or SPECIMENS	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQUOT DERIVATIVE	ALIQUOT SUB	INSTRUCTIONS FOR PROCESSING LAB			
	Cervical Tissue Biopsy (CVB) for PK	NON	BPS	N/A	Pre (without biopsy) and Post (with biopsy) weights are needed. Collect 2 biopsies, each placed in a pre-weighed cryovial. Flash-freeze (dry ice/ethanol bath). Store at $\leq -70^{\circ}\text{C}$.			
	Biopsy 1 Collection Time ____:____ hour : min				Post-weight ____.____ mg	Pre-weight ____.____ mg	Net Weight ____.____ mg	Freezing Time ____:____ hour : min
	Biopsy 2 Collection Time ____:____ hour : min				____.____ mg	____.____ mg	____.____ mg	____:____ hour : min

Pelvic Exam Checklist

Cervical Specimens (10.8)

- Cervical Biopsy FOR PD (10.8.3)
 - Required at Day 56 or Day 91/PUEV, before ring removal depending on randomization schedule Two biopsies, one per cryovial
 - *LDMS Tracking Sheet*
 - Collection time: *LDMS tracking sheet, Cervical Specimen Storage CRF*
 - Sample preparation for immediate testing
 - Cryovial containing biopsy medium (made weekly, from local PD lab)

# of TUBES or SPECIMENS	PRIMARY SPECIMEN	PRIMARY ADDITIVE	ALIQUOT DERIVATIVE	ALIQUOT SUB	INSTRUCTIONS FOR PROCESSING LAB	
	Cervical Tissue Biopsy (CVB) For PD Collection Time ____:____ <u>hour</u> : <u>min</u>	BTM	BPS	N/A	Collect 2 biopsy, place in a cryovial tube containing 1 mL of chilled transport medium. Transport to Virology and Pharmacodynamic Laboratory within 30 minutes!	
					_____ PD Lab Receiving Staff	_____ Receiving time

Intravaginal Ring Storage

- Remnant drug analysis (Section 10.7.10)
- Day 91/PUEV
 - Collect samples before removing ring
- Prepare rings for storage
 - Ex: Ring that is removed prematurely and not re-inserted
 - Ex: Participant returns ring
- Procedure to prepare for storage
 - Gently clean with alcohol pad until no visible mucus or CVF
 - Set the VR on paper towel and allow to air dry
 - Place in resealable foil pack (original packaging)
 - Biohazard label must be on the packaging after used IVR placed in it.
 - Storage, -80°C
 - DISCUSSION: Where will the foil pack be stored for 3 months?

Shipping instructions, batched for end of study

◆ To MTN LC

- Plasma archive
- CVL for PD
- CVL Biomarkers
- CVF Biomarkers
- qPCR
- Used vaginal rings
- Serum for HSV 1/2 Testing

◆ To JHU CPAL

- Plasma for PK
- Rectal Swab for PK
- Vaginal Swab for PK
- Cervical Biopsy for PK
- CVL for PK

◆ To CONRAD or CONRAD-designated lab

- CVF for Anti-HSV Activity

◆ To local PD lab

- Cervical Biopsy for PD

Supplies Provided by MTN LC

- Sterile polyester-tipped swabs
 - vaginal smear, swabs for PK
- Slides with frosted ends and cases
 - vaginal smear
- Swabs and cryovials for qPCR
- Swabs and cryovials for biomarkers
- Starplex Starswab Anaerobic Transporters
- Starplex Starswab Double Swab Dry Transporters
- Cryovials for VAG, REC, and CX biopsy PK

Questions?



Hillier Laboratory