



MTN-036

Laboratory Training

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Objectives

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

Resources

- MTN LC: Magee-Womens Research Institute
 - Lorna Rabe 412-641-6041 lrabe@mwri.magee.edu
 - May Beamer: 412-641-6026 mbeamer@mwri.magee.edu
 - Wayne Hall, Ted Livant
- SSP Section 10: Lab Considerations
 - Most current version
 - www.mtnstopshiv.org
 - Lab SSP Tables

Specimen Management slide 1

- Specimen Labeling (Section 9.2, page 9-4)
 - SCHARP-provided participant ID (PTID) label.
 - 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 9.3, page 9-4)
 - 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

- Specimen Handling and Chain of Custody SOP
 - Local Lab Requisitions
 - Laboratory Data and Management System (LDMS) Tracking Sheet

LDMS (Section 10.4)

- Samples stored for MTN LC
 - Tables 10-3 and 10-4
- LDMS Tracking Sheet only:
 - On tracking sheet, freeze times to ensure within storage parameters
 - Pre and post weights for vaginal and rectal swabs, cx biopsy
 - Make sure post weight is larger
- LDMS Log In:
 - Collection times for PK samples, plasma archive
 - Net Weight for CVF PK, Rectal PK or CX Biopsy PK
 - The average weight for vag PK is ~81 mG (65-115 mG)

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

per local lab requirements

- 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 (Section 10.6.2)
 - Required at Screening and Day 91/PUEV
 - If indicated, monthly visits
 - *Local Laboratory Results CRF*

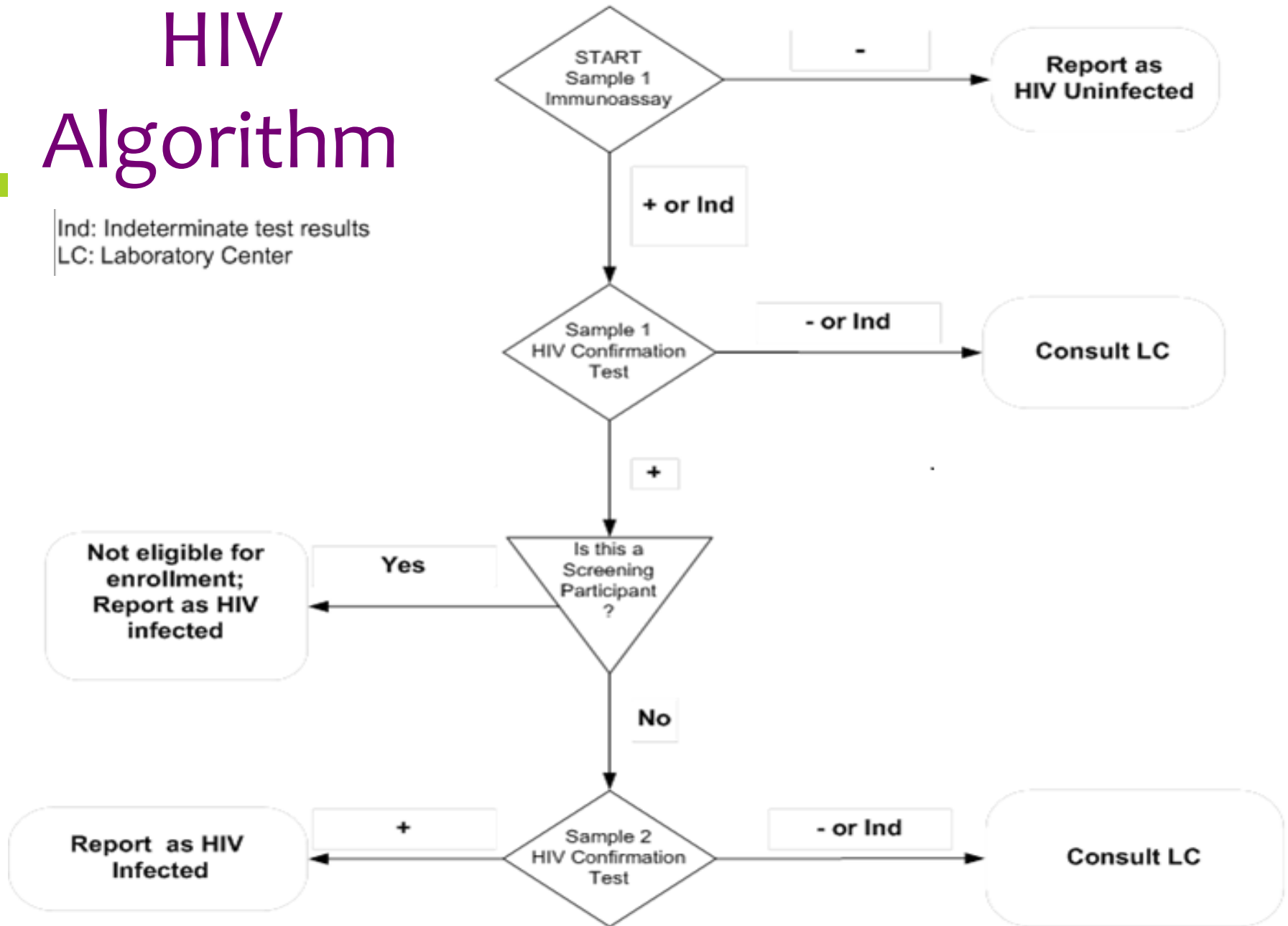
Blood Specimens slide 2

per local lab requirements

- Syphilis Serology (Section 10.6.4)
 - Required at Screening
 - If clinically indicated at follow-up visits (see Table 10-1)
 - *STI Test Results CRF*
- HIV-1 Testing (Section 10.6.3)
 - Required at Screening, Enrollment, Day 91/PUEV
 - If clinically indicated, follow-up visits
 - *HIV Results CRF, HIV Confirmation CRF*
 - HIV Testing Algorithm

HIV Algorithm

Ind: Indeterminate test results
LC: Laboratory Center



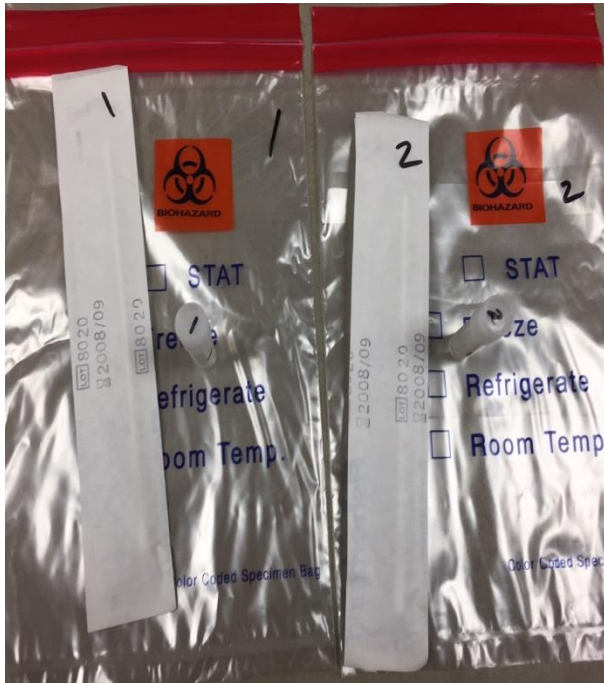
Blood Specimens, Stored for MTN LC

- Plasma archive (Section 10.6.5)
 - *LDMS Tracking Sheet, Specimen Storage CRF*
 - Required at Enrollment
 - If indicated at follow-up, SAMPLE 2 in HIV Algorithm
 - viral load and HIV drug resistance confirmation by MTN Virology
 - Storage: 1.5-mL aliquots; $\leq -70^{\circ}\text{C}$ within 24 hrs (4°C) or 4 hrs (RT°)
- Plasma for PK (Section 10.6.6)
 - *LDMS Tracking Sheet, Specimen Storage CRF*
 - Enrollment: Timer towards 1 HR time point starts when ring is inserted
 - Single time points, Days 28 and 56
 - Before ring removal for 28-day rings
 - Day 91/PUEV: 0 HR before ring removed
 - Timer towards 1 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

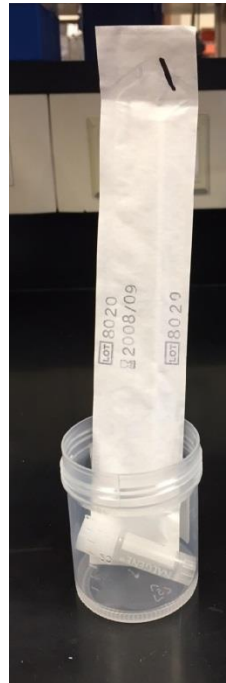
Rectal Fluid Swab for PK

- Enrollment (4-hours post insertion), Days 3, 7, 14, 28, 91 (prior to ring removal) and Final Contact visits.
- Pre-weight of packaged swab, cryovial
 - Place SCHARP labels prior to pre-weight
- Collection with anoscope
 - Within 30 minutes of blood collection
 - Hold swab against mucosa, 2 minutes
 - Time on *LDMS Tracking Sheet*, *Specimen Storage CRF*
- Post-weight
 - Place swab packaging, swab shaft into specimen bag
- Storage within 2 hrs, $\leq -70^{\circ}\text{C}$

Weighing Rectal and Vaginal Swabs for PK



Also have backup kits,
already weighed



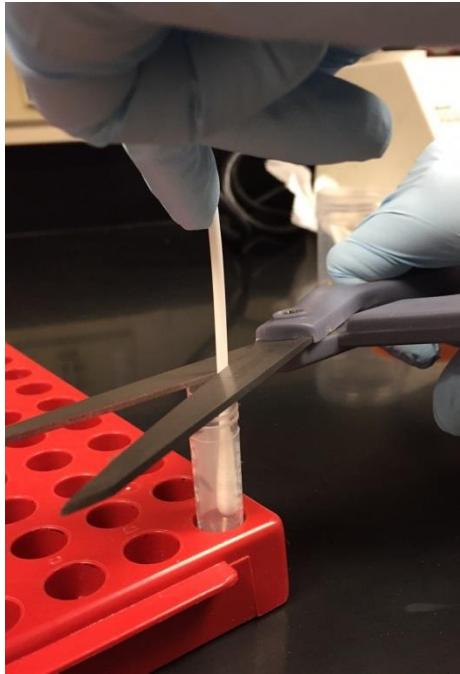
Don't throw
wrapper or swab
shaft in garbage



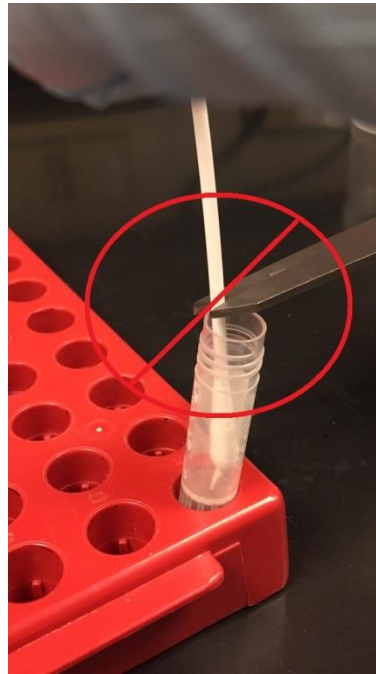
Weigh
everything



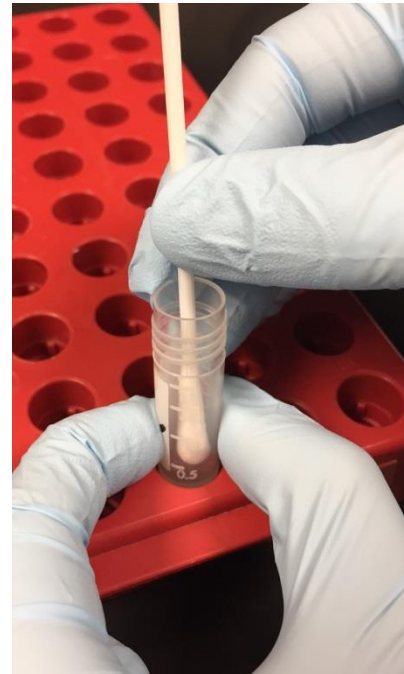
Cutting vs Bending Swab



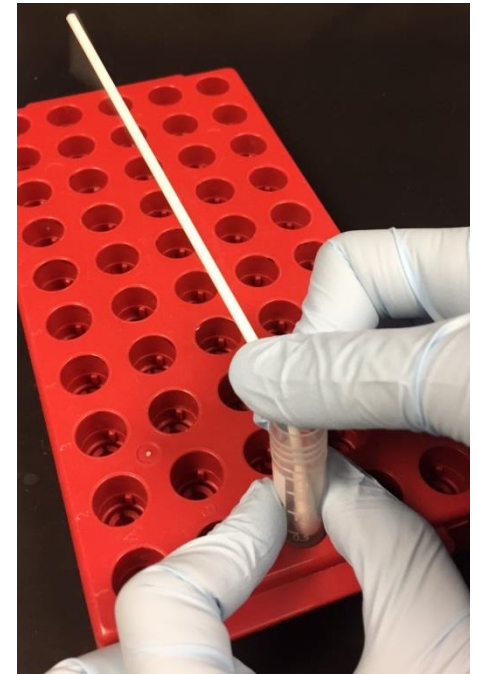
Easier cut



No leverage

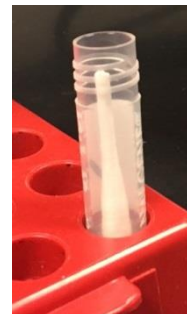


Raise swab



Thumb over opening

Raise swab before cutting or bending for lid to close properly



Vaginal Swab for PK_{slide 1} (10.7.6)

- Blood, Rectal swab (if applicable) for PK collected first
- Collected prior to speculum insertion
- All collected within 30 minutes time
- Similar weighing procedure as rectal fluid swab
 - Pre- & post-weights, net weights: *LDMS Tracking Sheet*
- Record collection time, *LDMS Tracking Sheet* and *Cervical Specimen Storage CRF*
- Enrollment
 - Ring insertion, timer starts for 1-, 2-, and 4-HR collection time target points
 - If collection delayed, no bearing on next time point

Vaginal Swab for PK slide 2 (10.7.6)

- Single time point
 - Days 1, 2, 3, 7, 14, 28, 56, Final Contact
- Days 28 and 56
 - Participants randomized to 28-day (25-mG) ring
 - Order of collection: Blood, Rectal swab, Vag PK samples before ring removal
- PUEV/Day 91
 - 0-HR, prior to ring removal
 - Same rules as enrollment above
 - If VR removed prior to visit, collect only single time point with blood for PK

Pelvic Exam Checklist slide 1

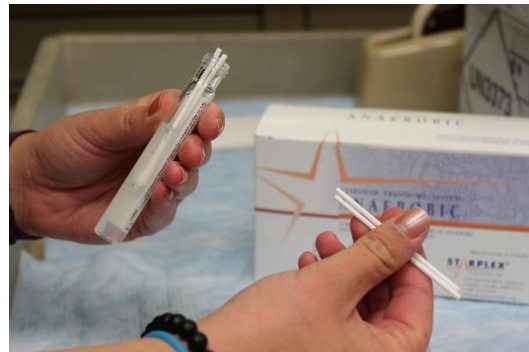
Vaginal Specimens

- *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 1

Quantitative Culture (10.7.3)

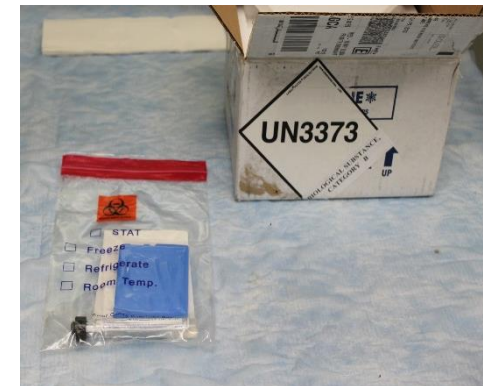
- Starplex Starswab Anaerobic Collection Kit, including 2 Dacron Swabs
 - Slowly insert swab into gel (swab halfway through gel)



Pelvic Exam Checklist slide 2

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 2

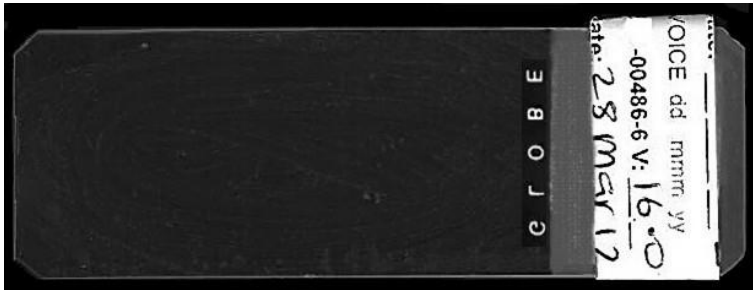
Vaginal Specimens

- 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 3

Vaginal Specimens

- Gram stain (Section 10.7.1)
 - Required at Enrollment, monthly visits
 - One swab, two slides



- *Specimen Storage CRF and LDMS Tracking Sheet*
- Storage
 - Primary VAG Gram Stain set
 - Duplicate VAG Gram Stain Set

Pelvic Exam Checklist

Cervical Specimens (10.8)

- Pap Test per local standard of care
 - If indicated at Screening, *STI Tests Results CRF*
- Cervical Biopsy
 - Required at Day 28 and PUEV/Day 91, before ring removal
 - Two biopsies, one per cryovial
 - Pre-weigh cryovials 1 and 2, post weight with biopsy, net weight
 - *LDMS Tracking Sheet*
 - Collection time: *LDMS tracking sheet, Cervical Specimen Storage CRF*
 - Sample preparation for storage
 - Immediate placement of cryovial in dry ice ethanol bath or liquid nitrogen
 - Storage @ $\leq -70^{\circ}\text{C}$

Pelvic Exam Checklist

Cervicovaginal Lavage (CVL)

- Collection procedure (10.7.7)
 - 10-mL normal saline
 - Collect CVL with syringe, place into 15-mL conical tube.
 - Cervical Specimen Storage CRF
- Collection time: *LDMS tracking sheet, Cervical Specimen Storage CRF*
 - Required at Enrollment prior to ring insertion;
 - Days 28 and 56 prior to ring removal;
 - PUEV/Day 91 4-HR post ring removal (speculum re-inserted)

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
 - 1 aliquot for CVL for PD
 - 1 aliquot for CVL for PK
 - 2 aliquots in CVL for biomarker box
 - Remainder in boxes labeled CVL supernatant

Intravaginal Ring Storage (10.7.8)

- Remnant content analysis
- *Ring Insertion Removal CRF*
- Prepare ring for storage if ring that is removed prematurely is not re-inserted.
- Days 28, 56, and 91/PUEV
 - Collect all PK samples BEFORE IVR removal.
- Safety from aerosol
- Mold-prevention: Must be dry before bagged
- Storage in amber bag

Shipping instructions, batched for end of study

◆ To MTN LC

- Plasma archive
- CVL for PD
- CVL Biomarkers
- qPCR
- Used vaginal rings

◆ To JHU CPAL

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

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
- Starplex Starswab Anaerobic Transporters
- Cryovials for VAG, REC, and CX PK
- Amber zippit bag: IVR



Questions?