MTN-038
Laboratory Training

May Beamer, Michele Austin
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://mntstopshiv.org/research/studies/mtn-038
  – Lab SSP Tables
Specimen Management (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.
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 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 (SSP Table 10-1)

<table>
<thead>
<tr>
<th>Blood Specimens</th>
<th>Visit 1 SCR</th>
<th>Visit 2 ENR (Day 0)</th>
<th>Visit 3 (Day 1)</th>
<th>Visit 4 (Day 7)</th>
<th>Visit 5 (Day 14)</th>
<th>Visit 6 (Day 28)</th>
<th>Visit 7 (Day 42)</th>
<th>Visit 8 (Day 56)</th>
<th>Visit 9 PUEV/Early Termination (Day 91)</th>
<th>Visit 10 Final Contact (Day 92)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1/2 testing</td>
<td>X</td>
<td>X</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>X</td>
</tr>
<tr>
<td>Plasma for archive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AST/ALT</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>CBC with differential and platelets</td>
<td>X</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>X</td>
<td>*</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>X</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>X</td>
<td>*</td>
</tr>
<tr>
<td>Hep B surface antigen</td>
<td>X</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syphilis serology</td>
<td>X</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>HSV 1/2 serology</td>
<td>X</td>
<td></td>
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<td></td>
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<tr>
<td>TFV levels</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* TFV levels measured in 0-, 4-, and 8-HR post-dose samples.
• 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; ≤ -70°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

START Immunoassay

- or Ind
+ or Ind

Sample 1
HIV Confirmation Test

- or Ind
+

Is this a Screening Participant?

No

Sample 2
HIV Confirmation Test

- or Ind
+

Report as HIV infected

Yes

Not eligible for enrollment

Follow-up visit:
Sample 2 AND additional blood for plasma collected for MTN LC

Contact MTN LC

Contact MTN LC

Report as HIV uninfected

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; ≤-70°C within 8 hours
    - Primary Plasma PK set; Backup Plasma PK set

<table>
<thead>
<tr>
<th># of TUBES or SPECIMENS</th>
<th>PRIMARY SPECIMEN</th>
<th>PRIMARY ADDITIVE</th>
<th>ALIQUOT DERIVATIVE</th>
<th>ALIQUOT SUB</th>
<th>INSTRUCTIONS FOR PROCESSING LAB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0-HR</strong> Blood (BLD) PK</td>
<td><strong>Collection Time</strong></td>
<td><strong>EDT</strong> (purple top)</td>
<td><strong>PL1</strong> or <strong>PL2</strong></td>
<td><strong>N/A</strong></td>
<td><strong>Single spin:</strong> 1300xg, 10 minutes <strong>OR</strong> <strong>Double spin:</strong> 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 ≤ -70°C within 8 hours of collection.</td>
</tr>
</tbody>
</table>
# Blood Specimens, Stored for MTN LC

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Primary Specimen</th>
<th>Primary Derivative</th>
<th>Aliquot Derivative</th>
<th>Aliquot Sub additive/derivative</th>
<th>Other Specimen ID</th>
<th># of Aliquots</th>
<th>Aliquot Volume</th>
<th>Units</th>
<th>Time or Weights</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma for Archive or Confirmatory Test</td>
<td>BLD</td>
<td>EDT</td>
<td>PL1 (single spin)</td>
<td>N/A</td>
<td>CON (follow-up)</td>
<td>2-5</td>
<td>≥1.5 mL in 2-mL cryovials</td>
<td>mL</td>
<td>--</td>
</tr>
<tr>
<td>Plasma for PK (TFV)</td>
<td></td>
<td></td>
<td>PL2 (double spin)</td>
<td></td>
<td>PK</td>
<td>2-5</td>
<td>≥1.0 mL in 2-mL cryovial</td>
<td>mL</td>
<td>See 10.4.2</td>
</tr>
<tr>
<td>Serum for HSV-1/2 Testing</td>
<td>BLD</td>
<td>NON</td>
<td>SER</td>
<td>N/A</td>
<td></td>
<td>1-2</td>
<td>≥1.0 mL in 2-mL cryovial</td>
<td>mL</td>
<td>--</td>
</tr>
</tbody>
</table>
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, ≤-70°C

<table>
<thead>
<tr>
<th># of TUBES or SPECIMENS</th>
<th>PRIMARY SPECIMEN</th>
<th>PRIMARY ADDITIVE</th>
<th>ALIQUOT DERIVATIVE</th>
<th>ALIQUOT SUB</th>
<th>INSTRUCTIONS FOR PROCESSING LAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-HR Rectal Fluid (REC) for PK</td>
<td>NON</td>
<td>SWB</td>
<td>N/A</td>
<td>Pre (before sample collection) and Post (after sample collection) weights are needed. May be kept on ice until frozen. Freeze at ≤-70°C within 2 hours of collection.</td>
<td></td>
</tr>
<tr>
<td>Collection Time</td>
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<tr>
<td>[ ] : [ ] : [ ] hour : min</td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>
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*

<table>
<thead>
<tr>
<th># of TUBES or SPECIMENS</th>
<th>PRIMARY SPECIMEN</th>
<th>PRIMARY ADDITIVE</th>
<th>ALIQUOT DERIVATIVE</th>
<th>ALIQUOT SUB</th>
<th>INSTRUCTIONS FOR PROCESSING LAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-HR Vaginal Swab (VAG) for PK</td>
<td><strong>NON</strong></td>
<td><strong>SWB</strong></td>
<td><strong>N/A</strong></td>
<td></td>
<td>Pre (before sample collection) and Post (after sample collection) weights are needed. May be kept on ice until frozen. Freeze at ≤ -70°C within 2 hours of collection.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Collection Time</th>
<th>Post- weight</th>
<th>Pre- weight</th>
<th>Net Weight</th>
<th>Freezing Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>____ : ____</td>
<td>____ . ____</td>
<td>____ . ____</td>
<td>____ . ____</td>
<td>____ : ____</td>
</tr>
<tr>
<td>hour : min</td>
<td>mg</td>
<td>mg</td>
<td>mg</td>
<td>hour : min</td>
</tr>
</tbody>
</table>
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*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Primary Specimen</th>
<th>Primary Derivative</th>
<th>Aliquot Derivative</th>
<th>Aliquot Sub additive/derivative</th>
<th>Other Specimen ID</th>
<th># of Aliquots</th>
<th>Aliquot Volume</th>
<th>Units</th>
<th>Time or Weights</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVF/vaginal swab for PK</td>
<td>VAG</td>
<td>NON</td>
<td>SWB</td>
<td>N/A</td>
<td>PK</td>
<td>1</td>
<td>variable</td>
<td>mG</td>
<td>See 10.4.2</td>
</tr>
<tr>
<td>CVF for anti-HSV-2 activity</td>
<td>CVF</td>
<td>CTK</td>
<td>SWB</td>
<td>N/A</td>
<td>HSV-VG</td>
<td>2</td>
<td>1</td>
<td>Each</td>
<td>See 10.4.2</td>
</tr>
<tr>
<td>CVF for Biomarkers</td>
<td>CVF</td>
<td>NON</td>
<td>SWB</td>
<td>N/A</td>
<td></td>
<td>1</td>
<td>1</td>
<td>Each</td>
<td>See 10.4.2</td>
</tr>
</tbody>
</table>

- 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 ≤-70°C storage
  - LDMS tracking sheet, Cervical Specimen Storage CRF

<table>
<thead>
<tr>
<th>Sample</th>
<th>Primary Specimen</th>
<th>Primary Derivative</th>
<th>Aliquot Derivative</th>
<th>Aliquot Sub additive/derivative</th>
<th>Other Specimen ID</th>
<th># of Aliquots</th>
<th>Aliquot Volume</th>
<th>Units</th>
<th>Time or Weights</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVF/vaginal swab for PK</td>
<td>VAG</td>
<td>NON</td>
<td>SWB</td>
<td>N/A</td>
<td>PK</td>
<td>1</td>
<td>variable</td>
<td>mL</td>
<td>See 10.4.2</td>
</tr>
<tr>
<td>CVF for anti-HSV-2 activity</td>
<td>CVF</td>
<td>CTK</td>
<td>SWB</td>
<td>N/A</td>
<td>HSV-VG</td>
<td>2</td>
<td>1</td>
<td>Each</td>
<td>See 10.4.2</td>
</tr>
<tr>
<td>CVF for Biomarkers</td>
<td>CVF</td>
<td>NON</td>
<td>SWB</td>
<td>N/A</td>
<td></td>
<td>1</td>
<td>1</td>
<td>Each</td>
<td>See 10.4.2</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Visit 1 SCR</th>
<th>Visit 2 ENR (Day 0)</th>
<th>Visit 3 (Day 1)</th>
<th>Visit 4 (Day 7)</th>
<th>Visit 5 (Day 14)</th>
<th>Visit 6 (Day 28)</th>
<th>Visit 7 (Day 42)</th>
<th>Visit 8 (Day 56)</th>
<th>Visit 9 PUEV/Early Termination (Day 91)</th>
<th>Visit 10 Final Contact (Day 92)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVL for PK</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CVL for PD and biomarkers</td>
<td>Xα</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
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</tr>
</tbody>
</table>

- 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 @ ≤-70°C within 2 hours of collection

<table>
<thead>
<tr>
<th># of TUBES or SPECIMENS</th>
<th>PRIMARY SPECIMEN</th>
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<th>ALIQUOT DERIVATIVE</th>
<th>ALIQUOT SUB</th>
<th>INSTRUCTIONS FOR PROCESSING LAB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cervicovaginal</td>
<td>NSL</td>
<td>FLD</td>
<td>N/A</td>
<td>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 ≤-70°C within 2 hours of collection.</td>
</tr>
<tr>
<td></td>
<td>Lavage (CVL)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Collection Time</td>
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<td></td>
<td>hour : min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CEN</td>
<td>NSL</td>
<td></td>
<td></td>
<td>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 ≤-70°C within 2 hours of collection.</td>
</tr>
</tbody>
</table>

Freezing Time
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

<table>
<thead>
<tr>
<th>Cervical biopsies for PK</th>
<th>Cervical biopsies for PK and PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit 1 SCR</td>
<td>Visit 2 ENR (Day 0)</td>
</tr>
<tr>
<td>X±</td>
<td>X±</td>
</tr>
</tbody>
</table>
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 @ ≤-70°C

<table>
<thead>
<tr>
<th># of TUBES or SPECIMENS</th>
<th>PRIMARY SPECIMEN</th>
<th>PRIMARY ADDITIVE</th>
<th>ALIQUOT DERIVATIVE</th>
<th>ALIQUOT SUB</th>
<th>INSTRUCTIONS FOR PROCESSING LAB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cervical Tissue Biopsy (CVB) for PK</td>
<td></td>
<td></td>
<td></td>
<td>Pre (without biopsy) and Post (with biopsy) weights are needed. Collect 2 biopsies, each placed in a pre-weighted cryovial. Flash-freeze (dry ice/ethanol bath). Store at ≤-70°C.</td>
</tr>
<tr>
<td></td>
<td>Biopsy 1 Collection Time</td>
<td>NON</td>
<td>BPS</td>
<td>N/A</td>
<td>Post-weight</td>
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<td></td>
<td>_hour : _min</td>
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<td></td>
<td>Biopsy 2 Collection Time</td>
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<td></td>
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<td></td>
<td>_hour : _min</td>
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</table>
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)

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<th>ALIQUOT DERIVATIVE</th>
<th>ALIQUOT SUB</th>
<th>INSTRUCTIONS FOR PROCESSING LAB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cervical Tissue</td>
<td>BTM</td>
<td>BPS</td>
<td>N/A</td>
<td>Collect 2 biopsy, place in a</td>
</tr>
<tr>
<td></td>
<td>Biopsy (CVB) For PD</td>
<td></td>
<td></td>
<td></td>
<td>cryovial tube containing 1 mL</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>of chilled transport medium.</td>
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<td></td>
<td></td>
<td></td>
<td>Transport to Virology and</td>
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<td>Pharmacodynamic Laboratory</td>
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<td>within 30 minutes!</td>
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</tbody>
</table>

PDU 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

<table>
<thead>
<tr>
<th>To MTN LC</th>
<th>To JHU CPAL</th>
<th>To CONRAD or CONRAD-designated lab</th>
<th>To local PD lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Plasma archive</td>
<td>• Plasma for PK</td>
<td>• CVF for Anti-HSV Activity</td>
<td>• Cervical Biopsy for PD</td>
</tr>
<tr>
<td>• CVL for PD</td>
<td>• Rectal Swab for PK</td>
<td></td>
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</tr>
<tr>
<td>• CVL Biomarkers</td>
<td>• Vaginal Swab for PK</td>
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</tr>
<tr>
<td>• CVF Biomarkers</td>
<td>• Cervical Biopsy for PK</td>
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</tr>
<tr>
<td>• qPCR</td>
<td>• CVL for PK</td>
<td></td>
<td></td>
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<tr>
<td>• Used vaginal rings</td>
<td></td>
<td></td>
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<tr>
<td>• Serum for HSV 1/2 Testing</td>
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</tbody>
</table>
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