MTN 026
Laboratory Training
Enrollment Visit

Pam Kunjara, MT ASCP
MTN Laboratory Center
Magee-Womens Research Institute
Pittsburgh, PA
Objectives

- Overview of Lab testing
- Collection of Pelvic Samples
- Collection of Anorectal Specimens
- Specimen Management
- Q&A
### Overview of Lab Testing by Visit

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

★ As indicated, ♦ Randomized assigned time points
# Overview of Lab Testing by Visit

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

★ 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

START
Immunoblot

-/- or EIA -
Report as HIV uninfected

-/+ or +/EIA +

Not eligible for enrollment:
Report as HIV infected

Is this a Screening Participant?

Yes

No

HIV Confirmation Test

- or Ind
Consult Laboratory Center

Report as HIV infected

*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 flocked 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 ≤-70°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 ≤-70°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 ≤-70°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.
Note: Weights are recorded in equation format.

### LDMS Tracking Sheets

<table>
<thead>
<tr>
<th># of TUBES or SPECIMENS</th>
<th>PRIMARY SPECIMEN</th>
<th>PRIMARY ADDITIVE</th>
<th>ALIQUOT DERIVATIVE</th>
<th>ALIQUOT SUB ADD/DER</th>
<th>INSTRUCTIONS FOR PROCESSING</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rectal Swab – Microflora (REC)</td>
<td>NON</td>
<td>SWB</td>
<td>N/A</td>
<td>Time Frozen: _<strong><strong><strong>:</strong></strong></strong> Hour : Min</td>
</tr>
<tr>
<td></td>
<td>Collection Time:_<strong><strong>:</strong></strong>__ Hour : Min</td>
<td></td>
<td></td>
<td></td>
<td>Freeze at ≤-70°C within 2 hours of collection. Enter MF into Other Spec ID field of LDMS.</td>
</tr>
<tr>
<td></td>
<td>Rectal Swab – PK (REC)</td>
<td>NON</td>
<td>SWB</td>
<td>N/A</td>
<td>Time Frozen: _<strong><strong><strong>:</strong></strong></strong> Hour : Min</td>
</tr>
<tr>
<td></td>
<td>Collection Time:_<strong><strong>:</strong></strong>__ Hour : Min</td>
<td></td>
<td></td>
<td></td>
<td>Freeze at ≤-70°C within 2 hours of collection. Enter PK into Other Spec ID field of LDMS.</td>
</tr>
<tr>
<td></td>
<td>Rectal Sponge – Mucosal Immunology (REC)</td>
<td>NON</td>
<td>SPG</td>
<td>N/A</td>
<td>Time Frozen: _<strong><strong><strong>:</strong></strong></strong> Hour : Min</td>
</tr>
<tr>
<td></td>
<td>Collection Time:_<strong><strong>:</strong></strong>__ Hour : Min</td>
<td></td>
<td></td>
<td></td>
<td>Freeze at ≤-70°C within 2 hours of collection.</td>
</tr>
<tr>
<td></td>
<td>Rectal Enema – PD (REC)</td>
<td>NSL</td>
<td>FLD</td>
<td>N/A</td>
<td>Time Frozen: _<strong><strong><strong>:</strong></strong></strong> Hour : Min</td>
</tr>
<tr>
<td></td>
<td>Suspend in 0.5ml of normal saline and freeze at ≤-70°C within 8 hours of collection.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PEN</td>
<td>NSL</td>
<td></td>
<td></td>
<td>Store supernatants in aliquots of 1.0 ml. Freeze at ≤-70°C within 8 hours of collection.</td>
</tr>
<tr>
<td></td>
<td>Rectal Biopsies – PD (FSR)</td>
<td>BTM</td>
<td>BPS</td>
<td>N/A</td>
<td>Store supernatants in aliquots of 1.0 ml. Freeze at ≤-70°C within 8 hours of collection.</td>
</tr>
<tr>
<td></td>
<td>Collection Time:_<strong><strong>:</strong></strong>__ Hour : Min</td>
<td></td>
<td></td>
<td></td>
<td>1 _<strong><strong><strong>:</strong></strong></strong> = _<strong><strong><strong>:</strong></strong></strong> mg</td>
</tr>
<tr>
<td></td>
<td>2 _<strong><strong><strong>:</strong></strong></strong> = _<strong><strong><strong>:</strong></strong></strong> mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 _<strong><strong><strong>:</strong></strong></strong> = _<strong><strong><strong>:</strong></strong></strong> mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Any Questions?